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(71) Applicant *(for all designated States except US)*: **THE BURNHAM INSTITUTE** [US/US]; 10901 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FUKUDA, Minoru** [US/US]; 2818 Passy Avenue, San Diego, CA 92122 (US). **YEH, Jiunn-Chern** [—/US]; 3128 Via Alicante Drive #E, La Jolla, CA 92037 (US). **HIRAOKA, Nobuyoshi** [JP/US]; 7405 Charmant Drive #2206, San Diego, CA 92122 (US).

(74) Agents: **GASHLER, Andrea, L.** et al.; Campbell & Flores LLP, 7th floor, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).

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(54) Title: THE MECA-79 ANTIGEN AND RELATED METHODS

(57) Abstract: The present invention provides the structure of the MECA-79 antigen and methods of treating L-selectin-mediated conditions by modulating enzymes that are required for formation of this antigen.

IDENTIFICATION OF THE MECA-79 ANTIGEN AND RELATED METHODS OF TREATING L-SELECTIN-MEDIATED CONDITIONS

This application was made with government support under CA 71932, CA 48737 and CA 33000 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to lymphocyte homing and pathologies involving chronic or acute inflammation mediated by L-selectin and, more specifically, to identification of the L-selectin ligand antigen, MECA-79.

BACKGROUND INFORMATION

In mammals, lymphocytes circulate in the vascular and lymphatic compartments, allowing maximum exposure of lymphocytes to foreign pathogens. Lymphocytes leave the vascular compartment at lymph nodes, traverse the lymphatic organs, and then return to the vascular system. This directed flow of lymphocytes is dependent on carbohydrate ligands present on specialized endothelial cells, known as high endothelial venules (HEV; Arbones et al., Immunity 1:247-260 (1994)). Although the structure of these carbohydrate ligands is unknown, lymphocyte binding to HEV depends on sialic acid on HEV and can be inhibited by fucosylated sulfated oligosaccharides (Rosen and Bertozzi, Curr. Biol.

261:261-264 (1996)). The homing receptor on lymphocytes is L-selectin, which contains an amino-terminal carbohydrate-binding domain similar to that of the hepatic lectin. Carbohydrate-binding activity of these lectins is calcium-dependent, and they are therefore termed "C-type" lectins (Drickamer, "Molecular Structure of Animal Lectins" in Fukuda and Hindsgaul (Eds), Molecular Glycobiology Oxford University Press: Oxford, U.K. (1994)). Counterreceptors (ligands) on HEV capture circulating lymphocytes via L-selectin-dependent adhesion, leading to transmigration. It has been shown that L-selectin is required for this process (Arbones et al., *supra*, 1994).

The HEV-expressed counterreceptors (ligands) for L-selectin have thus far eluded molecular identification. Consistent with the presence of a C-type lectin domain at the amino terminus of L-selectin, all of the ligands identified to date contain carbohydrate-based recognition determinants. In mouse lymph nodes, two such ligands have been identified as GlyCAM-1 and CD34, both of which are sialomucins (Lasky et al., Cell 69:927-938 (1992); Baumhueter et al., Science 262:436-438 (1993)). CD34 is a type I transmembrane glycoprotein, whereas GlyCAM-1 is a secreted molecule that lacks a transmembrane domain. Additionally, MadCAM-1, which contains a mucin domain in addition to Ig-like domains, can function as a ligand for L-selectin in Peyer's patches (Berg et al., Nature 366:695-698 (1993); and Bargatze et al., Immunity 3:99-108 (1995)). Four human glycoprotein ligands have been biochemically identified, and two of these have been cloned as CD34 and podocalyxin (Berg et al., J. Cell Biol. 114:343-349 (1991); Puri et

al., J. Cell Biol. 131:261-270 (1995); and Sassetti et al., J. Exp. Med. 187:1965-1975 (1998)). All of the human and murine ligands are sialomucin-like, (Puri et al., *supra*, 1995), and CD34 and podocalyxin have a similar overall domain structure (Figure 1) with significant sequence homology in their cytoplasmic domains (Sassetti et al., *supra*, 1998). Notably, only certain glycoforms react with L-selectin. For example, naturally occurring forms of GlyCAM-1, MadCAM-1, CD34 and podocalyxin exist which fail to bind L-selectin due to the absence of necessary post-translational modification (Berg et al., Nature 366:695-698 (1993); Puri et al., *supra*, 1995; Sassetti et al., *supra*, 1998; and Dowbenko et al., J. Clin. Invest. 92:952-960 (1993)). Thus, although CD34 and podocalyxin are widely distributed on vascular endothelium, a limited number of vessels (including HEV) express L-selectin-reactive glycoforms (Sassetti et al., *supra*, 1998; and Baumhueter et al., Blood 84:2554-2565 (1994)).

GlyCAM-1 and CD34 were originally identified as L-selectin ligands in extracts of mouse lymph nodes using a recombinant L-selectin/IgG chimera (Lasky et al., *supra*, 1992; Baumhueter et al., *supra*, 1993; and Imai et al., J. Cell Biol. 113:1213-1221 (1991)). Furthermore, a monoclonal antibody, MECA-79, stains HEV in mouse lymph nodes and blocks both lymphocyte attachment to HEV *in vitro* and short-term homing of lymphocytes to lymph nodes *in vivo* (Streeter et al., Nature 331:41-43 (1988)). The MECA-79 monoclonal is remarkable in that it reacts with HEV across a wide range of species including mouse and human (Girard et al., FASEB J. 12:603-612 (1998)). Significantly, MECA-79 and L-selectin/IgG stain the same

complex of glycoproteins in mouse and human lymphoid organs (Sassetti et al., *supra,* 1998; and Hemmerich et al., J. Exp. Med. 180:2219-2226 (1994)). This complex of four or more glycoproteins defined by reactivity with MECA-79 is known as peripheral lymph node addressin (PNAd). Although the structure of the MECA-79 antigen has eluded identification, the epitope is believed to be sulfated (Hemmerich et al., *supra,* 1994) and, in particular, to include a GlcNAc-6-sulfate modification (Kimura et al., Proc. Natl. Acad. Sci. 96:4530-4535 (1999)). Furthermore, previous characterization indicates that the MECA-79 epitope is independent of sialylation and fucosylation (Hemmerich et al., *supra,* 1994; and Maly et al., Cell 86:643-653 (1996). Nevertheless, the physiologically relevant sulfated structures necessary for L-selectin ligand activity remain to be identified.

L-selectin and its ligands are implicated in lymphocyte recruitment in a variety of chronic inflammatory diseases, and L-selectin ligand activity including MECA-79 expression is induced on microvascular venular endothelium in rheumatoid arthritis, lymphocytic thyroiditis, and inflammatory bowel diseases such as Crohn's disease and ulcerative colitis (Michie et al., Am. J. Pathol. 143:1688-1698 (1993); and Salmi et al., Gastroenterology 106:596-605 (1994)). Increased MECA-79 expression also is associated with nonobese diabetes in the mouse and with transplant rejection (Hanninen et al., J. Clin. Invest. 92:2509-2515 (1993); and Toppila et al., Am. J. Pathol. 155:1303-1310 (1999)).

Methods of controlling L-selectin activity would be desirable in order to reduce inflammatory responses mediated by L-selectin. Such methods could be used to treat or prevent conditions such as acute or chronic inflammation; allograft rejection; or tumor metastasis. However, methods of specifically controlling L-selectin activity await elucidation of the sulfated carbohydrate structure on L-selectin ligands, and identification of the enzymes that manufacture the L-selectin ligand carbohydrate determinants.

Thus, there is a need for identification of the L-selectin ligand carbohydrate structure and identification of the enzyme or enzymes that produce this structure. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated β1,3GnT, or an active fragment thereof, under conditions that allow addition of core 1 GlcNAc linkages to the acceptor molecule, where the β1,3GnT or active fragment thereof directs expression of a MECA-79 antigen. A β1,3GnT useful for modifying an acceptor molecule according to a method of the invention can have, for example, substantially the amino acid sequence of human β1,3GnT (SEQ ID NO: 2) or substantially the amino acid sequence of murine β1,3GnT (SEQ ID NO: 4).

The invention also provides a method of treating or preventing an L-selectin-mediated condition

in a subject by reducing the expression or activity of a β1,3GnT that directs expression of a MECA-79 antigen. In a method of the invention, the expression or activity of a β1,3GnT can be reduced, for example, by administering to a subject an oligosaccharide L-selectin antagonist that inhibits the binding of L-selectin to a MECA-79 antigen. Such an L-selectin antagonist can contain, for example, the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc or the oligosaccharide NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1, or, in another embodiment, multimers of one or both of these oligosaccharides. In a further embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject inhibitory antibody material that specifically binds β1,3GnT. In yet a further embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject a β1,3GnT antisense nucleic acid molecule that has, for example, at least 20 nucleotides complementary to SEQ ID NO: 1 or SEQ ID NO: 3. In another embodiment, a method of the invention is practiced by reducing the expression or activity of a β1,3GnT that directs expression of a MECA-79 antigen in combination with reducing the expression or activity of L-selectin sulfotransferase-2 (LSST-2) in the subject.

The present invention also provides an isolated L-selectin antagonist containing an extended core 1 structure which includes the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc. In a further embodiment, the invention provides an isolated L-selectin antagonist containing the oligosaccharide NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNA

cαl. In yet another embodiment, an isolated L-selectin antagonist of the invention contains multimers of one or both the the oligosaccharides Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc or Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc.

The present invention also provides an isolated polypeptide which contains an amino acid sequence encoding a L-selectin sulfotransferase-2 (LSST-2), or an active fragment thereof, that directs expression of a MECA-79 antigen in Chinese hamster ovary (CHO) cells. An isolated polypeptide of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6).

The present invention further provides substantially purified antibody material that specifically binds a LSST-2 that directs expression of a MECA-79 antigen in CHO cells. Such antibody material, which can be polyclonal or monoclonal antibody material, specifically binds, for example, human LSST-2 having the amino acid sequence SEQ ID NO: 6.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic acid sequence encoding a LSST-2 or an active fragment thereof that directs expression of a MECA-79 antigen in CHO cells. An isolated nucleic acid molecule of the invention can encode, for example, a LSST-2 that has substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) and can be, for example, SEQ ID NO: 5. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding a

LSST-2 or active fragment thereof that directs expression of a MECA-79 antigen in CHO cells. In one embodiment, such a vector is a mammalian expression vector.

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 5, shown in Figure 4. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 5. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated LSST-2, or an active fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule, where the LSST-2 or active fragment thereof directs expression of a MECA-79 antigen in CHO cells. A LSST-2 useful for modifying an acceptor molecule according to a method of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) or an active fragment thereof.

The invention also provides a method of treating or preventing an L-selectin-mediated condition in a subject by reducing the expression or activity of a LSST-2 that directs expression of a MECA-79 antigen in CHO cells. In one embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject inhibitory antibody material that specifically binds LSST-2. In another embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject a LSST-2 antisense nucleic acid molecule that has, for example, at least 20 nucleotides complementary to SEQ ID NO: 5.

The invention also provides an isolated polypeptide that contains an amino acid sequence encoding substantially the amino acid sequence of intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) or an active fragment thereof. Such a polypeptide of the invention can have, for example, substantially the amino acid sequence of SEQ ID NO: 8.

In addition, the invention also provides substantially purified antibody material that specifically binds an isolated polypeptide having an amino acid sequence encoding substantially the amino acid sequence of I-GlcNAc6ST or an active fragment thereof. Such antibody material, which can be polyclonal or monoclonal antibody material, specifically binds, for example, I-GlcNAc6ST having the amino acid sequence SEQ ID NO: 8.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic

acid sequence encoding an I-GlcNAc6ST or an active fragment thereof. An isolated nucleic acid molecule of the invention can encode, for example, an I-GlcNAc6ST having substantially the amino acid sequence of murine I-GlcNAc6ST (SEQ ID NO: 8) and can be, for example, SEQ ID NO: 7. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding an I-GlcNAc6ST or active fragment thereof. In one embodiment, such a vector is a mammalian expression vector.

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 7, shown in Figure 10. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 7. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated I-GlcNAc6ST, or an active

fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a model of lymph node HEV ligands for L-selectin. Four sialomucins recognized by MECA-79 are shown. GlyCAM-1, CD34, and Sgp200 have been identified in mouse lymph node. CD34, podocalyxin and Sgp200 have been identified in human tonsils. The complex, defined by purification with MECA-79, is denoted the peripheral lymph node addressin (PNAd). The cDNA encoding Sgp200 (sulfated glycoprotein of 200 kd) has yet to be cloned. White circles designate posttranslational modifications including sialylation, fucosylation, and sulfation. CD34 and podocalyxin share the same overall structural organization, each having an amino-terminal mucin domain, a presumed globular domain with cysteines, a transmembrane domain, and homologous cytoplasmic tails.

Figure 2 shows the human β1,3GnT nucleotide sequence (SEQ ID NO: 1) and predicted amino acid sequence (SEQ ID NO: 2).

Figure 3 shows the murine β1,3GnT nucleotide sequence (SEQ ID NO: 3) and predicted amino acid sequence (SEQ ID NO: 4).

Figure 4 shows the human L-selectin sulfotransferase-2 (hLSST-2) nucleotide sequence (SEQ ID NO: 5) and predicted amino acid sequence (SEQ ID NO: 6).

Figure 5 shows a CLUSTALW alignment of mouse β3GalT-I, -II, -III and -IV and mouse β3GnT proteins. Conserved residues are shaded. White arrows mark the positions of the cysteine residues conserved among β3GalT proteins. The black arrow shows the position of the cysteines conserved in the five proteins.

Figure 6 shows *in vitro* substrate specificity of human β1,3GnT.

Figure 7 shows MECA-79 staining of transfected CHO/CD34 cells.

Figure 8 shows the results of a rolling experiment performed with four stably transfected CHO cell lines. Open circles represent the CHO/CD34/FT7/hLSST-2 cell line. Open squares represent the CHO/CD34/FT7/hLSST-2/C2GnT-L cell line. Filled squares represent the CHO/CD34/FT7/hLSST-2/core 1 extension β1,3GnT cell line. Filled circles represent the CHO/CD34/FT7/hLSST-2/C2GnT-L/core 1 extension β1,3GnT line cell.

Figure 9 shows inhibition of anti-MECA-79 antibody binding to MECA-79-reactive CD34 chimeric proteins by synthetic oligosaccharides including sialylated and sialylated, fucosylated forms of the extended core 1 structure.

Figure 10 shows the murine intestinal-GlcNAc 6-sulfotransferase (I-GlcNAc6ST) nucleotide sequence (SEQ ID NO: 7) and predicted amino acid sequence (SEQ ID NO: 8).

DETAILED DESCRIPTION OF THE INVENTION

Lymphocyte homing is important for the surveillance of foreign pathogens. Extravasation of lymphocytes in peripheral lymph nodes is mediated through L-selectin binding to L-selectin ligands, sulfated sialyl Lewis^x present on high endothelial venules (HEV). Recently cloned L-selectin ligand sulfotransferases (LSST or HEC-GlcNac6ST) form core 2-based selectin ligand functional in rolling assays (Hiraoka et al., Immunity 11:79-89 (1999), and Bistrup et al., J. Cell. Biol. 145:899-910 (1999)). The expression of LSST is highly restricted to HEV, while the sulfotransferase GlcNAc6ST is more widely present and less specific in acceptor substrate requirement.

Analysis of core 2 GnT-leukocyte type knockout mice has indicated that lymphocyte homing and expression of MECA-79 antigen persist even after the gene for the leukocyte type core 2 GnT has been inactivated (Ellies et al., Immunity 9:881-890 (1998)). Structural analysis of L-selectin ligands in HEV of the knockout mice demonstrated that the major oligosaccharides remaining are based on extended core 1 structure such as NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1→R. As disclosed herein, a novel β1,3-N-acetylglucosaminyl-transferase has been isolated that extends core 1 and forms GlcNAcβ1→3Galβ1→3GalNAcα1→R. As further disclosed herein, human L-selectin sulfotransferase-2 (hLSST-2), is unique in the ability to produce, when co-transfected into CHO cells together with β1,3-GnT, NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]

β1→3Galβ1→3GalNAcα1→R, resulting in expression of the MECA-79 epitope. As further disclosed herein, oligosaccharides produced in CHO cells expressing both human β1,3GnT and human LSST-2 support L-selectin-mediated lymphocyte rolling (see Example III). These results demonstrate that 6-sulfo sialyl Lewis X structures on core 1 or core 2 oligosaccharides can serve as L-selectin ligands on high endothelial venules.

As further disclosed herein in Example IV, several synthetic oligosaccharides were compared for the ability to inhibit binding of anti-MECA-79 antibody to the MECA-79 antigen produced in the media of CHO/CD34/FT7/hLSST-2/core 1 β1,3GnT cells. As shown in Figure 9, only the synthetic oligosaccharide with the 6-S-extended core 1 structure (Galβ1→4(SO_3→6) GlcNAcβ1→3Galβ1→3GalNAc) was able to inhibit antibody binding to MECA-79, defining Galβ1→4(SO_3→6) GlcNAcβ1→3Galβ1→3GalNAc as a minimal MECA-79 epitope. Sialylated or sialylated and fucosylated forms of the 6-sulfo extended core 1 structure (NeuNAcα2→3Galβ1→4[Fucα1→3(sulfo→6)]GlcNAcβ1→3Galβ1→3GalNAcα1→octyl) also were efficient inhibitors of antibody binding (Figure 9). As further disclosed herein, the 6-sulfo group was absolutely required, since non-sulfated, extended core 1 did not inhibit MECA-79 antibody binding (Figure 9). In addition, the terminal galactose residue in the *N*-acetyllactosaminyl core 1 was part of the epitope, since the agalacto form required more than a 10 fold greater concentration to achieve equivalent inhibition (Figure 9). An absolute requirement for core 1 structure was also demonstrated, since sulfated *N*-acetyllactosamine lacking a core 1

structure did not show detectable inhibition (Figure 9). These results indicate that the minimum epitope of MECA-79 is the sulfated, extended core1 structure Galβ1→4(sulfo→6)GlcNAcβ1→3Galβ1→3GalNAcα1→R, and that the sialylated and sialylated, fucosylated forms (6-sulfo sLe^x in extended core 1) retain MECA-79 reactivity.

Thus, the present invention is directed to the long-awaited discovery of the structure and minimum epitope of the L-selectin ligand, MECA-79, and to identification of a β1,3-N-acetylglucosaminyl transferase (β1,3GnT) and a human sulfotransferase (hLSST-2) that can produce this ligand when co-expressed in CHO cells. These discoveries provide a basis for diagnosing and treating L-selectin-mediated conditions, including acute and chronic inflammation, transplant rejection and tumor metastasis.

The present invention relates to an isolated polypeptide which contains an amino acid sequence encoding a β1,3GnT, or an active fragment thereof, that directs expression of a MECA-79 antigen in CHO cells. Such an isolated polypeptide can have, for example, substantially the amino acid sequence of human β1,3GnT (SEQ ID NO: 2) or substantially the amino acid sequence of murine β1,3GnT (SEQ ID NO: 4).

The term "β1,3-N-acetylglucosaminyl-transferase," as used herein, is synonymous with "β1,3GnT" and means an enzyme that catalyzes the β1→3 linkage of a N-acetylglucosamine (GlcNAc) residue to an acceptor molecule. A β1,3GnT useful in the invention is a core 1 extension enzyme and, therefore, catalyzes the

β1→3 linkage of a GlcNAc residue to the core 1 structure Galβ1→3GalNAc→R.

A β1,3GnT that directs expression of a MECA-79 epitope can have, for example, substantially the amino acid sequence of the human β1,3GnT shown in Figure 2 as SEQ ID NO: 2 or substantially the amino acid sequence of the murine β1,3GnT shown in Figure 3 as SEQ ID NO: 4. Human β1,3GnT polypeptide (SEQ ID NO: 2) is a type II membrane protein of 352 amino acids. Human β1,3GnT (SEQ ID NO: 2) shares 66.5% amino acid identity with murine β1,3GnT (SEQ ID NO: 4). Regions highly conserved between human and murine β1,3GnT are present, for example, at amino acids 158 to 245, 263 to 322 and 330 to 361 of SEQ ID NO: 2. As disclosed in Example IB, human β1,3GnT (SEQ ID NO: 2) forms the MECA-79 antigen when expressed with L-selectin ligand sulfotransferase-2 in Chinese hamster ovary (CHO) cells. Thus, such a β1,3 GnT is characterized, in part, by the ability to direct expression of a MECA-79 antigen.

The mouse monoclonal antibody, MECA-79, stains HEV in mouse lymph nodes and blocks lymphocyte attachment to HEV *in vitro* as well as short-term homing of lymphocytes to lymph nodes *in vivo* (Streeter et al., *supra,* 1988). Furthermore, the MECA-79 monoclonal antibody reacts with HEV across a variety of species and stains the same complex of glycoproteins in mouse and human lymphoid organs (Girard et al., *supra,* 1998; Sassetti et al., *supra,* 1998; Hemmerich et al. *supra,* 1994). Thus, while the carbohydrate-based recognition determinants on the HEV-expressed L-selectin ligands CD34, podocalyxin, Sgp200 and GlyCAM-2 remain unknown,

these L-selectin ligands contain the MECA-79 antigen (Hemmerich, *supra,* 1994).

As used herein, the term "MECA-79 antigen" means a carbohydrate-containing epitope that specifically reacts with the MECA-79 monoclonal antibody described in Hemmerich, *supra,* 1994. An exemplary MECA-79 antigen is provided herein as Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc. The phrase "directs expression of a MECA-79 antigen" refers to production of a carbohydrate-containing epitope that specifically reacts with the MECA-79 monoclonal antibody. It is understood that an enzyme "directs expression of a MECA-79 antigen" only under the appropriate conditions. Such conditions include availability of a core 1 acceptor molecule and an appropriate donor molecule and further include the presence of one or more additional enzymes. Human β1,3GnT together with the human sulfotransferase LSST-2, but not other sulfotransferases, directs expression of the MECA-79 antigen in CHO cells.

The invention provides a method of treating or preventing an L-selectin-mediated condition in a subject by reducing the expression or activity of a β1,3GnT that directs expression of a MECA-79 antigen. If desired, a method of the invention can be practiced by reducing the expression or activity of a β1,3GnT that directs expression of a MECA-79 antigen in combination with reducing the expression or activity of L-selectin sulfotransferase-2 (LSST-2) in the subject.

As used herein, the term "L-selectin-mediated condition" means any pathology or disorder involving the

L-selectin ligand, MECA-79. Such an L-selectin-mediated condition generally can be, for example, acute or chronic inflammation, allograft rejection, or tumor metastasis. An L-selectin-mediated condition also can be, for example, organ transplant rejection, which is typically accompanied by an influx of lymphocytes into the graft. For example, in a rat model of acute cardiac allograft rejection, Toppila et al. demonstrated the induction of L-selectin ligands including MECA-7 on flat-walled venules and capillaries within rejecting cardiac allograft (Toppila et al., Am. J. Pathol. 155:1303-1310 (1999)). Toppila et al. further observed a correlation between the staining intensity of L-selectin ligands on vessels and the severity of acute rejection of heart allografts in humans. L-selectin-mediated conditions further can include rheumatoid arthritis; inflammatory bowel diseases such as Crohn's disease and ulcerative colitis; inflammatory disorders of the skin such as allergic contact dermatitis, psoriasis and Lichen planus; lymphomas; chronic pneumonia; delayed-type hypersensitivity reactions; diabetes; and hyperplastic thymus, each of which are characterized by expression of MECA-79 in HEV-like vessels (Rosen, Am. J. Pathol. 155:1013-1020 (1999); see, also, Table 1). It is understood that these and other conditions of acute or chronic inflammation, allograft rejection or tumor metastasis can be an "L-selectin-mediated" condition that can be treated according to a method of the invention.

Table 1		
L-selectin-mediated conditions		
Organ	**Disease process**	**Reference**
Synovium	Rheumatoid arthritis	Michie et al., Am. J. Path. 143:1688-1698 (1993); Van Dinther-Jansses et al., J. Rheum. 17-11-17 (1990)
Gut	Crohn's disease	Salmi et al.., Gastroenterology 106:596-605 (1994); Duijvestijn et al., J. Immunol. 138:713-719 (1987)
Gut	Ulcerative colitis	Salmi et al., Eur. J. Immunol. 22:835-843 (1992)
Skin	Cutanteous sites of inflammation such as allergic contact dermatitis, psoriasis and lichen planus	Michie et al., *supra*, 1993; Arvilommi et al., Eur. J. Immunol. 26:825-833 (1996)
Skin	Cutaneous lymphomas	Michie et al., *supra*, 1993
Lung	Chronic interstitial pneumonia	
Skin	Delayed-type hypersensitivity reaction	Mackay et al., Eur. J. Immunol. 22:835-843 (1992)
Pancreas	Diabetes	Hanninen et al., J. Clin. Invest. 92:2509-2515 (1993)
Thymus	Hyperplastic thymus	Michie et al., Am. J. Path. 147:412-421 (1995)

The term "reducing the expression or activity" as used herein to a β1,3GnT, means that the amount of functional β1,3GnT polypeptide or activity is diminished

in the subject in comparison with the amount of functional β1,3GnT polypeptide in an untreated subject. Similarly, when used in reference to LSST-2 expression or activity, the term "reduced" means that the amount of functional LSST-2 polypeptide or activity is reduced in the treated subject as compared to an untreated subject. Thus, the term "reduced," as used herein, encompasses the absence of a β1,3GnT that directs expression of a MECA-79 antigen or a LSST-2, as well as protein expression that is present but reduced as compared to the level of β1,3GnT or LSST-2 expression in an untreated subject. Furthermore, the term reduced refers to suppressed refers to β1,3GnT or LSST-2 protein expression that is diminished throughout the entire domain of β1,3GnT or LSST-2 expression, or to expression that is reduced in some part of the β1,3GnT or LSST-2 expression domain, provided that expression of the MECA-79 antigen is decreased.

As used herein, the term "reduced" also encompasses an amount of β1,3GnT or LSST-2 polypeptide that is equivalent to wild type β1,3GnT or LSST-2 expression, but where the β1,3GnT or LSST-2 polypeptide has a reduced level of activity. For example, mutations within the catalytic domain of β1,3GnT or LSST-2 that reduce glucosaminyltransferase activity or sulfotransferase activity, respectively, are encompassed within the meaning of the term "reduced."

The present invention relates, in part, to the use of carbohydrate-based drugs for treatment of an L-selectin-mediated condition such as rheumatoid arthritis, inflammatory bowel disease or diabetes.

Carbohydrate drugs are well known in the art and include, for example, Acarbose, a maltotetrose analog for treatment of diabetes, which acts as a competitive inhibitor of sucrase and α-amylase (Bayer AG; Balfour and McTavish, Drugs 46:1025 (1993). Other carbohydrate drugs include Relenza™ (GG-167, zanamivir), a sialic acid analog for treatment of influenza which is a selective inhibitor of viral neuramidases (Glaxo Wellcome/Biota; Hayden et al., JAMA 275:295 (1996), and SYNSORB Pk™, an oligosaccharide conjugate for treatment of *E. coli* 0157.H7 infection developed by SYNSORB Biotech. Additional carbohydrate-based drugs are well known in the art (see, for example, Dumitrui (Ed.), Polysaccharides in Medicinal Applications Dekker, New York (1996)).

In one embodiment, the invention provides a method of treating or preventing an L-selectin-mediated condition in a subject by administering to the subject an oligosaccharide L-selectin antagonist that inhibits the binding of L-selectin to a MECA-79 antigen. Such an L-selectin antagonist can contain, for example, the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc or the oligosaccharide NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1 or, in another embodiment, multimers of one or both of these oligosaccharides.

As disclosed herein, the MECA-79 epitope has the structure Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc and is based on a core 1 structure. As further disclosed herein, an L-selectin ligand contains the MECA-79 related structure NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1. The term "core 1," as used herein, means the core structure Galβ1→3GalNAc→R. In conformance

with accepted carbohydrate and chemical nomenclature, "Gal" means galactose; "GalNAc" means N-acetylgalactosamine; "GlcNAc" means N-acetylglucosamine; "SO_3" means sulfate; and "NeuNAc" means N-acetylneuraminate, also known as sialic acid. "R" can be a serine or threonine residue of a peptide or protein or, for example, an octyl, O-methyl, *p*-nitrophenol, amino pyridine, or other convenient moiety.

The term "oligosaccharide," as used herein, means a linear or branched carbohydrate that consists of from 2 to about 50 monosaccharide units joined by means of glycosidic bonds. The monosaccharide units of an oligosaccharide are polyhydroxy alcohols containing either an aldehyde or a ketone group. An oligosaccharide can have, for example, up to 5, 10, 20, 30, 40 or 50 monosaccharide units. It is understood that "an oligosaccharide L-selectin antagonist" may have other non-carbohydrate components in addition to its carbohydrate component.

An L-selectin antagonist also can be a glycoconjugate or glycomimetic based on the structure Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc or NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1. Thus, an L-selectin antagonist of the invention can be a synthetic glycoconjugate or glycomimetic that retains the ability to inhibit binding of L-selectin to a MECA-79 antigen (Yarema and Bertozzi, Curr. Opin. Chem. Biol. 2:49-61 (1998); Dumitrui, *supra*, 1996). Multivalent glycoconjugates are particularly useful L-selectin antagonists of the invention.

As disclosed herein, the MECA-79 epitope is formed, in part, by a core 1 extension enzyme (β1,3GnT) which catalyzes the β1→3 linkage of a GlcNAc residue to the core 1 structure (Galβ1→3GalNAc→R) and has the structure Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc. Based on this discovery, the present invention provides an oligosaccharide L-selectin antagonist containing an extended core 1 structure which includes the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc. In one embodiment, an isolated L-selectin antagonist contains the oligosaccharide NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1. In another embodiment, an L-selectin antagonist contains multimers of one or both of the oligosaccharides Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc and NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1. In addition to the structural features set forth above, an L-selectin antagonist inhibits L-selectin activity, for example, by competing for binding to physiological L-selectin ligand. L-selectin antagonists also include variants of these structures which cannot accept a GlcNAc residue at the 3 position of galactose, such as structures in which C-3 of galactose is deoxy; or variants in which GlcNAc contains a 6-dehydro group. Other L-selectin antagonists can be core 1 structure derivatives which cannot accept a GlcNAc residue at the 3 position of galactose.

In a further embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject inhibitory antibody material that specifically binds β1,3GnT. In yet a further embodiment, an L-selectin-mediated condition is treated or prevented by

administering to the subject a β1,3GnT antisense nucleic acid molecule that has, for example, at least 20 nucleotides complementary to SEQ ID NO: 1 or SEQ ID NO: 3.

The present invention also provides an isolated polypeptide which contains an amino acid sequence encoding a L-selectin sulfotransferase-2 (LSST-2), or an active fragment thereof, that directs expression of a MECA-79 antigen in Chinese hamster ovary (CHO) cells. An isolated polypeptide of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6).

As used herein, the term "isolated" means a polypeptide or nucleic acid molecule that is in a form that is relatively free from contaminating lipids, polypeptides, nucleic acids or other cellular material normally associated with the nucleic acid molecule or polypeptide in a cell.

A LSST-2 polypeptide can have substantially the amino acid sequence of SEQ ID NO: 6. Thus, an LSST-2 polypeptide of the invention can be the naturally occurring human LSST-2 (SEQ ID NO: 6), or a related polypeptide having substantial amino acid sequence similarity to this sequence. Such a related polypeptide typically exhibits greater sequence similarity to human LSST-2 than to other sulfotransferases such as murine LSST, and includes isotype variants, alternatively spliced forms and species homologs of the amino acid sequence shown in Figure 4. As used herein, the term "LSST-2" generally describes polypeptides having an amino

acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid sequence identity with SEQ ID NO: 6, said amino acid identity determined with CLUSTALW using the BLOSUM 62 matrix with default parameters, provided that such a polypeptide is able to produce the MECA-79 antigen when expressed in CHO cells under the appropriate conditions. The previously described murine polypeptide, LSST (Hiraoka et al., *supra,* 1999), which is not able to form the MECA-79 antigen when co-transfected into CHO cells with hβ1,3GnT, therefore is not a LSST-2 polypeptide of the invention.

The present invention also provides active fragments of a LSST-2 polypeptide. As used herein, the term "active fragment" means a polypeptide fragment having substantially the amino acid sequence of a portion of a LSST-2 that directs expression of a MECA-79 antigen in CHO cells, provided that the fragment retains the sulfotransferase activity of the parent polypeptide as well as the ability to direct expression of a MECA-79 antigen in CHO cells. An active fragment of LSST-2 can have, for example, substantially the amino acid sequence of a portion of human LSST-2 (SEQ ID NO:6). Sulfotransferase activity can be assayed, for example, as described in Hiraoka et al., Immunity 11:79-89 (1999). Activity in directing expression of a MECA-79 antigen can be assayed as set forth in Example IB.

As used herein, the term "substantially the amino acid sequence," when used in reference to a LSST-2

polypeptide or an active fragment thereof, is intended to mean a sequence as shown in Figure 4, or a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an amino acid sequence that has substantially the amino acid sequence of a human LSST-2 polypeptide (SEQ ID NO: 6) can have one or more modifications such as amino acid additions, deletions or substitutions relative to the amino acid sequence of SEQ ID NO: 6, provided that the modified polypeptide retains substantially the ability to direct expression of a MECA-79 antigen in CHO cells, as described further below.

Thus, it is understood that limited modifications can be made to a human LSST-2 polypeptide or another polypeptide of the invention (see below), or to an active fragment thereof without destroying its biological function. A modification can be, for example, an addition, deletion, or substitution of one or more conservative or non-conservative amino acid residues; substitution of a compound that mimics amino acid structure or function; or addition of chemical moieties such as amino or acetyl groups. The activity of a modified LSST-2 polypeptide or fragment thereof can be assayed by transfecting an encoding nucleic acid molecule into CHO cells and assaying for expression of MECA-79 as disclosed herein.

A particularly useful modification of a polypeptide of the invention, or fragment thereof, is a modification that confers, for example, increased stability. Incorporation of one or more D-amino acids is a modification useful in increasing stability of a

polypeptide or polypeptide fragment. Similarly, deletion or substitution of lysine can increase stability by protecting against degradation.

The present invention also provides substantially purified antibody material that specifically binds a LSST-2 that directs expression of a MECA-79 antigen in CHO cells. Such antibody material, which can be polyclonal or monoclonal antibody material, specifically binds, for example, human LSST-2 having the amino acid sequence SEQ ID NO: 6.

A LSST-2 polypeptide or polypeptide fragment can be useful to prepare substantially purified antibody material that specifically binds a LSST-2 which directs expression of a MECA-79 antigen in CHO cells. Such antibody material can be, for example, substantially purified polyclonal antiserum or monoclonal antibody material. The antibody material of the invention be useful, for example, in determining the level of LSST-2 polypeptide in a subject.

As used herein, the term "antibody material" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a specific binding activity for a LSST-2 polypeptide of at least about 1×10^5 M^{-1}. One skilled in the art would know that anti-LSST-2 antibody fragments such as Fab, $F(ab')_2$ and Fv fragments can retain specific binding activity for a LSST-2 polypeptide and, thus, are included within the definition of antibody material. In addition, the term "antibody material," as used herein, encompasses non-naturally occurring

antibodies and fragments containing, at a minimum, one V_H and one V_L domain, such as chimeric antibodies, humanized antibodies and single chain Fv fragments (scFv) that specifically bind a LSST-2 polypeptide. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced recombinantly or obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Borrebaeck (Ed.), Antibody Engineering (Second edition) New York: Oxford University Press (1995)).

Antibody material "specific for" a LSST-2 polypeptide, or that "specifically binds" a LSST-2 polypeptide, binds with substantially higher affinity to that polypeptide than to an unrelated polypeptide. The substantially purified antibody material of the invention also can bind with significantly higher affinity to a LSST-2 that directs expression of a MECA-79 antigen in CHO cells than to another sulfotransferase that does not direct expression of a MECA-79 antigen in CHO cells.

Anti-LSST-2 antibody material can be prepared, for example, using a LSST-2 fusion protein or a synthetic peptide encoding a portion of a LSST-2 polypeptide such as SEQ ID NO: 6 as an immunogen. One skilled in the art would know that purified LSST-2 polypeptide, which can be produced recombinantly, or fragments of LSST-2, including peptide portions of LSST-2 such as synthetic peptides, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides of LSST-2 can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin

(KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art are described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988)).

The term "substantially purified," as used herein in reference to antibody material, means that the antibody material is substantially devoid of polypeptides, nucleic acids and other cellular material which with an antibody is normally associated in a cell. The claimed antibody material that specifically binds an LSST-2 further is substantially devoid of antibody material of unrelated specificities, i.e. that does not specifically bind a LSST-2. The antibody material of the invention can be prepared in substantially purified form, for example, by LSST-2 affinity purification of polyclonal anti-LSST-2 antisera, by screening phage displayed antibodies against a LSST-2 polypeptide such as SEQ ID NO: 6, or as monoclonal antibodies prepared from hybridomas.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic acid sequence encoding a LSST-2 or an active fragment thereof that directs expression of a MECA-79 antigen in CHO cells. An isolated nucleic acid molecule of the invention can encode, for example, a LSST-2 that has substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) and can be, for example, SEQ ID NO: 5. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding a LSST-2 or active fragment thereof that directs expression

of a MECA-79 antigen in CHO cells. In one embodiment, such a vector is a mammalian expression vector.

The term "nucleic acid molecule" is used broadly to mean any polymer of two or more nucleotides, which are linked by a covalent bond such as a phosphodiester bond, a thioester bond, or any of various other bonds known in the art as useful and effective for linking nucleotides. Such nucleic acid molecules can be linear, circular or supercoiled, and can be single stranded or double stranded DNA or RNA or can be a DNA/RNA hybrid.

A sense or antisense nucleic acid molecule or oligonucleotide of the invention also can contain one or more nucleic acid analogs. Nucleoside analogs or phosphothioate bonds that link the nucleotides and protect against degradation by nucleases are particularly useful in a nucleic acid molecule or oligonucleotide of the invention. A ribonucleotide containing a 2-methyl group, instead of the normal hydroxyl group, bonded to the 2'-carbon atom of ribose residues, is an example of a non-naturally occurring RNA molecule that is resistant to enzymatic and chemical degradation. Other examples of non-naturally occurring organic molecules include RNA containing 2'-aminopyrimidines, such RNA being 1000x more stable in human serum as compared to naturally occurring RNA (see Lin et al., Nucl. Acids Res. 22:5229-5234 (1994); and Jellinek et al., Biochemistry 34:11363-11372 (1995)).

Additional nucleotide analogs also are well known in the art. For example, RNA molecules containing

2'-O-methylpurine substitutions on the ribose residues and short phosphorothioate caps at the 3'- and 5'-ends exhibit enhanced resistance to nucleases (Green et al., Chem. Biol. 2:683-695 (1995). Similarly, RNA containing 2'-amino- 2'-deoxypyrimidines or 2'-fluro-2'-deoxypyrimidines is less susceptible to nuclease activity (Pagratis et al., Nature Biotechnol. 15:68-73 (1997). Furthermore, L-RNA, which is a stereoisomer of naturally occurring D-RNA, is resistant to nuclease activity (Nolte et al., Nature Biotechnol. 14:1116-1119 (1996); Klobmann et al., Nature Biotechnol. 14:1112-1115 (1996). Such RNA molecules and methods of producing them are well known and routine (see Eaton and Piekern, Ann. Rev. Biochem. 64:837-863 (1995). DNA molecules containing phosphorothioate linked oligodeoxynucleotides are nuclease resistant (Reed et al., Cancer Res. 50:6565-6570 (1990). Phosphorothioate-3' hydroxypropylamine modification of the phosphodiester bond also reduces the susceptibility of a DNA molecule to nuclease degradation (see Tam et al., Nucl. Acids Res. 22:977-986 (1994), which is incorporated herein by reference). Furthermore, thymidine can be replaced with 5-(1-pentynyl)- 2'-deoxoridine (Latham et al., Nucl. Acids Res. 22:2817-2822 (1994). It is understood that nucleic acid molecules, including antisense molecules and oligonucleotides, containing one or more nucleotide analogs are encompassed by the invention.

The invention also provides vectors containing a nucleic acid molecule encoding a LSST-2. Such vectors can be cloning vectors or expression vectors and provide a means to transfer an exogenous nucleic acid molecule into a host cell, which can be a prokaryotic or

eukaryotic cell. Contemplated vectors include those derived from a virus, such as a bacteriophage, a baculovirus or a retrovirus, and vectors derived from bacteria or a combination of bacterial and viral sequences, such as a cosmid or a plasmid. The vectors of the invention can advantageously be used to clone or express LSST-2 or an active fragment thereof. Various vectors and methods for introducing such vectors into a host cell are described, for example, in Ausubel et al., Current Protocols in Molecular Biology John Wiley & Sons, Inc. New York (1999).

In addition to a nucleic acid molecule encoding a LSST-2 or active fragment thereof, a vector of the invention also can contain, if desired, one or more of the following elements: an oligonucleotide encoding, for example, a termination codon or a transcription or translation regulatory element; one or more selectable marker genes, such as an ampicillin, tetracycline, neomycin, hygromycin or zeomycin resistance gene, which is useful for selecting stable transfectants in mammalian cells; one or more enhancer or promoter sequences, which can be obtained, for example, from a viral, bacterial or mammalian gene; transcription termination and RNA processing signals, which are obtained from a gene or a virus such as SV40; an origin of replication such as an SV40, polyoma or *E. coli* origin of replication; versatile multiple cloning sites; and one or more RNA promoters such as a T7 or SP6 promoter, which allows for *in vitro* transcription of sense and antisense RNA.

In one embodiment, a vector of the invention is an expression vector. Expression vectors are well known

in the art and provide a means to transfer and express an exogenous nucleic acid molecule in a host cell. Contemplated expression vectors include vectors that provide for expression in a host cell such as a bacterial cell, yeast cell, insect cell, frog cell, mammalian cell or other animal cell. Such expression vectors include regulatory elements specifically required for expression of the DNA in a cell, the elements being located relative to the nucleic acid molecule encoding LSST-2 so as to permit expression thereof. The regulatory elements can be chosen to provide constitutive expression or, if desired, inducible or cell type-specific expression. Regulatory elements required for expression have been described above and include transcription and translation start sites and termination sites. Such sites permit binding, for example, of RNA polymerase and ribosome subunits. A bacterial expression vector can include, for example, an RNA transcription promoter such as the *lac* promoter, a Shine-Delgarno sequence and an initiator AUG codon in the proper frame to allow translation of an amino acid sequence.

Mammalian expression vectors can be particularly useful and can include, for example, a heterologous or homologous RNA transcription promoter for RNA polymerase binding, a polyadenylation signal located downstream of the coding sequence, an AUG start codon in the appropriate frame and a termination codon to direct detachment of a ribosome following translation of the transcribed mRNA. Commercially available mammalian expression vectors include pSI, which contains the SV40 enhancer/promoter (Promega; Madison, WI); pTarget™ and pCI, which each contain the cytomegalovirus (CMV)

enhancer/promoter (Promega); pcDNA3.1, a CMV expression vector (Invitrogen; Carlsbad, CA); and pRc/RSV, which contains Rous sarcoma virus (RSV) enhancer/promoter sequences (Invitrogen). In addition to these constitutive mammalian expression vectors, inducible expression systems are available, including, for example, an ecdysone-inducible mammalian expression system such as pIND and pVgRXR from Invitrogen. These and other mammalian expression vectors are commercially available or can be assembled by those skilled in the art using well known methods. An example of a eukaryotic expression vector of the invention is pcDNA1.1/LSST-2, described in Example II below.

The invention also provides a host cell containing a vector that includes a nucleic acid molecule encoding a LSST-2 or an active fragment thereof. Such a host cell can be used to replicate the vector and, if desired, to express and isolate substantially pure recombinant LSST-2 using well known biochemical procedures (see Ausubel, *supra*, 1999). In addition, a host cell of the invention can be used in an *in vitro* or *in vivo* method to transfer sulfate to an acceptor molecule. Such host cells can be chosen or transfected to additionally co-express one or more additional enzymes involved in oligosaccharide biosynthesis, for example, the core 1 extension enzyme, hβ1,3GnT. Such host cells can be used to prepare ligands having high affinity for the L-selectin glycoprotein receptor.

Host cells expressing LSST-2 or an active fragment thereof also can be used to screen for selective inhibitors of LSST-2 or for agents that selectively react

with a L-selectin ligand. These agents can be administered to a subject to prevent or treat an L-selectin-mediated condition as described further below.

Examples of host cells useful in the invention include bacterial, yeast, frog and mammalian cells. Various mammalian cells useful as host cells include, for example, mouse NIH/3T3 cells, CHO cells, COS cells and HeLa cells. In addition, mammalian cells obtained, for example, from a primary explant culture are useful as host cells. Additional host cells include non-human mammalian embryonic stem cells, fertilized eggs and embryos, which can be routinely used to generate transgenic animals, such as mice, which express the novel LSST-2 of the invention. Transgenic mice expressing LSST-2 can be used, for example, to screen for compounds that enhance or inhibit the MECA-79 producing activity of this enzyme. Methods for introducing a vector into a host including electroporation, microinjection, calcium phosphate, DEAE-dextran and lipofection methods well known in the art (see, for example, Ausubel, *supra*, 1999).

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 5, shown in Figure 4. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 5. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG.

An isolated antisense nucleic acid molecule can be useful to reduce LSST-2 expression, thereby treating or preventing an L-selectin-mediated condition in a subject. Antisense nucleic acid molecules can, for example, reduce mRNA translation or increase mRNA degradation and thereby suppress gene expression (see, for example, Galderisi et al., J. Cell Physiol. 181:251-257 (1999)). Methods of using antisense nucleic acid molecules as therapeutic agents are well known in the art (see Galderisi et al., *supra,* 1999; Alama et al., Pharmacol. Res. 36:171-178 (1997); and Temsamani et al., Biotechnol. Appl. Biochem. 26 (part 2):65-71 (1997))

The skilled artisan will recognize that effective reduction of LSST-2 expression depends upon the antisense nucleic acid molecule having a high percentage of homology with the endogenous LSST-2 locus, for example, the endogenous human locus SEQ ID NO: 5. A nucleic acid molecule encoding human LSST-2 (SEQ ID NO: 5) provided herein is useful in the antisense methods of the invention.

The homology requirement for effective suppression of gene expression using antisense methodology can be determined empirically. In general, a minimum of about 80-90% nucleic acid sequence identity is preferred for effective suppression of LSST-2 expression. More preferably, a nucleic acid molecule that is exactly homologous to the gene to be suppressed is used as an antisense nucleic acid molecule. Both antisense oligonucleotides of 20, 22, 25, 30, 35, 40 or more nucleotides, as well as antisense nucleic acid molecules

is expressed in a vector are contemplated for use in the antisense methods of the invention.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto.

Oligonucleotides of the invention can advantageously be used, for example, as primers for PCR or sequencing, as probes for research or diagnostic applications, and in therapeutic applications. An oligonucleotide of the invention can incorporate, if desired, a detectable moiety such as a radiolabel, fluorochrome, luminescent tag, ferromagnetic substance, or a detectable agent such as biotin, and used to detect expression of LSST-2 in a cell or tissue. Those skilled in the art can determine the appropriate length and nucleic acid sequence of a LSST-2 oligonucleotide for a particular application. An oligonucleotide of the invention contains a nucleotide sequence having, for example, at least, 10, 12, 14, 16, 18, 20, 25, 30, 35 or 40 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated LSST-2, or an active fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule, where the LSST-2

or active fragment thereof directs expression of a MECA-79 antigen in CHO cells. A LSST-2 useful for modifying an acceptor molecule according to a method of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) or an active fragment thereof. In a method of the invention, an isolated LSST-2 can add a sulfate to the 6-position of GlcNAc.

The term "acceptor molecule," as used herein, refers to a molecule that is acted upon, or "modified," by a protein having enzymatic activity. For example, an acceptor molecule can be a molecule that accepts the transfer of a sulfate due to the sulfotransferase activity of a LSST-2 polypeptide. An acceptor molecule can be in substantially pure form or in an impure form such as in a host cell or cellular extract. An acceptor molecule can be a naturally occurring molecule or a completely or partially synthesized molecule. An acceptor molecule can contain one or more sugar residues prior to modification and can be further modified to contain additional sugar residues. An acceptor molecule useful in the invention contains the core 1 structure (Galβ1-3GalNAc-R) and can be, for example, CD34 as disclosed herein. Additional acceptor molecules include podocalyxin, Sgp200 and GlyCAM-1.

In one embodiment, the invention provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated LSST-2 or an active fragment thereof in combination with an isolated β1,3GnT that directs expression of a MECA-79 antigen under conditions that allow addition of core 1 GlcNAc

linkages and sulfate to the acceptor molecule such that a MECA-79 antigen is formed. As disclosed herein, human β1,3GnT (SEQ ID NO: 2) and human LSST-2 (SEQ ID NO: 6) can be used together to modify a core 1 structure to produce the MECA-79 antigen, $Gal\beta1\rightarrow4(SO_3\rightarrow6)GlcNAc\beta1\rightarrow3Gal\beta1\rightarrow3GalNAc$, in CHO cells.

The invention also provides a method of treating or preventing an L-selectin-mediated condition in a subject by reducing the expression or activity of a LSST-2 that directs expression of a MECA-79 antigen in CHO cells. L-selectin-mediated conditions as well as techniques for reducing the expression or activity of an enzyme such as LSST-2 are described hereinabove.

As further disclosed herein in Example V, the mouse intestinal GlcNAc 6-sulfotransferase can, in combination with a β1,3GnT, form the MECA-79 antigen in Lec2 cells, but not in CHO cells. In these cells, which are defective in Golgi sialylation, more core 1 extension product is formed by the core 1 extension enzyme, β1,3GnT. Under these conditions, murine intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) adds enough sulfate to form the MECA-79 antigen. Thus, the invention also provides a novel nucleic acid molecule that contains a nucleic acid sequence encoding substantially the amino acid sequence of I-GlcNAc6ST or an active fragment thereof. An isolated nucleic acid molecule of the invention can encode, for example, substantially the amino acid sequence of SEQ ID NO: 8 and can be, for example, SEQ ID NO: 7. In one embodiment, an isolated nucleic acid molecule of the invention encodes

substantially the amino acid sequence of SEQ ID NO: 8, provided that the nucleic acid molecule is not AI115260.

The invention also provides an isolated polypeptide that contains an amino acid sequence encoding substantially the amino acid sequence of intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) or an active fragment thereof. Such a polypeptide of the invention can have, for example, substantially the amino acid sequence of SEQ ID NO: 8.

An I-GlcNAc6ST polypeptide has substantially the amino acid sequence of SEQ ID NO: 8. Thus, an I-GlcNAc6ST polypeptide of the invention can be the naturally occurring I-GlcNAc6ST (SEQ ID NO: 8), or a related polypeptide having substantial amino acid sequence similarity to this sequence. Such a related polypeptide includes isotype variants, alternatively spliced forms and species homologs of the amino acid sequence shown in Figure 10. As used herein, the term "I-GlcNAc6ST" generally describes polypeptides having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 75%, 80%, 85%, 90%, 95%, 97%, or 99% amino acid sequence identity with SEQ ID NO: 8, said amino acid identity determined with CLUSTALW using the BLOSUM 62 matrix with default parameters, provided that such a polypeptide is able to produce the MECA-79 antigen when expressed in Lec2 cells under the appropriate conditions. The previously described murine polypeptide, LSST (Hiraoka et al., *supra*, 1999) is not an I-GlcNAc6ST polypeptide of the invention.

The present invention also provides active fragments of an I-GlcNAc6ST polypeptide. As used herein, The term "active fragment," when used in reference to an I-GlcNAc6ST polypeptide, means a polypeptide fragment having substantially the amino acid sequence of a portion of an I-GlcNAc6ST, provided that the fragment retains the 6-sulfotransferase activity of the parent polypeptide as well as the ability to direct expression of a MECA-79 antigen when expressed in Lec2 cells. An active fragment can have, for example, substantially the amino acid sequence of a portion of murine I-GlcNAc6ST (SEQ ID NO:8). Sulfotransferase activity can be assayed, for example, as described in Hiraoka et al., Immunity 11:79-89 (1999). Activity in directing expression of a MECA-79 antigen can be assayed as set forth in Example IB.

Furthermore, the term "substantially the amino acid sequence," when used in reference to an I-GlcNAc6ST polypeptide or an active fragment thereof, is intended to mean a sequence as shown in Figure 10, or a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an amino acid sequence that has substantially the amino acid sequence of an I-GlcNAc6ST polypeptide (SEQ ID NO: 8) can have one or more modifications such as amino acid additions, deletions or substitutions relative to the amino acid sequence of SEQ ID NO: 8, provided that the modified polypeptide retains substantially 6-sulfotransferase activity as well as the ability to direct expression of a MECA-79 antigen in Lec2 cells (see Example V).

In addition, the invention also provides substantially purified antibody material that specifically binds an isolated polypeptide having an amino acid sequence encoding substantially the amino acid sequence of I-GlcNAc6ST or an active fragment thereof. Such antibody material, which can be polyclonal or monoclonal antibody material, specifically binds, for example, murine I-GlcNAc6ST having the amino acid sequence SEQ ID NO: 8. Thus, such antibody material includes polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a specific binding activity for an I-GlcNAc6ST polypeptide of at least about 1×10^5 M^{-1}. As set forth above, such antibody material includes Fab, $F(ab')_2$ and Fv fragments as well as chimeric and humanized antibodies and single chain Fv fragments (scFv) that specifically bind an I-GlcNAc6ST polypeptide of the invention.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic acid sequence encoding an I-GlcNAc6ST or an active fragment thereof. An isolated nucleic acid molecule of the invention can encode, for example, an I-GlcNAc6ST having substantially the amino acid sequence of murine I-GlcNAc6ST (SEQ ID NO: 8) and can be, for example, SEQ ID NO: 7. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding an I-GlcNAc6ST or active fragment thereof. In one embodiment, the vector is a mammalian expression vector. As set forth above, a variety of vectors, including cloning and expression vectors, and host cells are well known in the art.

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 7, shown in Figure 10. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 7. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG. An antisense nucleic acid molecule can have, for example, 20, 22, 25, 30, 35, 40 or more nucleotides.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto.

As set forth above, a sense or antisense nucleic acid molecule or oligonucleotide of the invention is a polymer of two or more nucleotides, which are linked by a covalent bond such as a phosphodiester bond, a thioester bond, or any of various other bonds known in the art as useful and effective for linking nucleotides. Furthermore, a nucleic acid molecule or oligonucleotide of the invention can contain one or more nucleic acid analogs (see above). An oligonucleotide of the invention contains a nucleotide sequence having, for example, at least, 10, 12, 14, 16, 18, 20, 25, 30, 35 or 40 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated I-GlcNAc6ST, or an active fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

CLONING AND CHARACTERIZATION OF THE HUMAN CORE 1 EXTENSION ENZYME, β1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE (β1,3GnT)

This example describes the cloning and characterization of human and murine β1,3-N-acetylglucosaminyltransferase (β1,3GnT).

A. Cloning and characterization of human β1,3GnT

Sequences homologous among β1,3-galactosyl-transferases and β1,3-N-acetylglucosaminyltransferases shown in Figure 5 (Zhou et al., Proc. Natl. Acad. Sci., USA 96:406-411 (1999)) were used as probes to search dbEST using the tblastn program. An EST clone (AB015630) containing a single open reading frame of 372 amino acids was obtained. Primers 5'-CTGGCTGGCCAGGATGAAGTATCTCC-3' (β1,3GnT-A1; SEQ ID NO: 9) and 5'-CCTGATGCTGACTCAGTAGATCTGTGTC-3' (β1,3GnT-A2AS; SEQ ID NO: 10) were designed based on EST AB015630. After amplification of single-stranded cDNA prepared from HT29 cells using the Thermoscript RT-PCR system (Gibco-BRL #11146-024; Baithersburg, MD), a 1.2 kb fragment

containing full-length coding sequence was isolated (see Figure 2). The 1.2 Kb fragment containing the full-length human β1,3GnT cDNA was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen) and designated pcDNA3.1/hβ1,3GnT-A.

In order to characterize the human β1,3GnT enzyme, a soluble form of the enzyme was prepared by amplifying amino acids 44 to 372 with PCR primers 5'-CGGGATCCCGAGGCCCTGGCCTGGCCCACTCC-3' (β1,3GnT-A-5'Bam; SEQ ID NO: 11) and 5'-GCTCTAGACTCAGTAGATCTGTGTCTGATTGC-3' (β1,3GnT-A-3'AS-Xba; SEQ ID NO: 12) and subsequently cloning the amplified fragment into the *BamHI* and *XbaI* sites of pcDNA3.1/HSH, a modified vector based on pCDNA3.1/Hydro (Invitrogen) and containing a signal peptide followed by a 6 histidine tag. This vector (4 μg) was transfected into Chinese hamster ovary (CHO) cells using lipofectamine PLUS (Gibco-BRL #10964-013). As a negative control, CHO cells were mock transfected with a vector lacking the β1,3GnT sequence.

Media from cells expressing the soluble enzyme or mock transfected were collected and concentrated essentially as described in Yeh et al., J. Biol. Chem. 274:3215-3221 (1999). For analysis of β1,3-galactosyltransferase activity, ^{3}H-UDP-galactose was used as the sugar nucleotide donor and GalNAc-α-pNP and GlcNAc-β-pNP were used as oligosaccharide acceptor molecules. For detection of β1,3-N-acetylglucosaminyltransferase (β1,3GnT) activity, ^{3}H-UDP-GlcNAc was used as the sugar nucleotide donor with the following oligosaccharide acceptor molecules: Galβ1,3Glc-β-pNP; core 1 pNP (Galβ1,3GalNAc-α-pNP);

core 2 pNP (Galβ1,3(GlcNAcβ1,6)GalNAc-α-pNP); Gal-α-pNP and Gal-β-pNP.

Supernatant from cells expressing the soluble enzyme or mock transfected was assayed for *in vitro* enzyme activity. As shown in Figure 6, concentrated medium from soluble enzyme transfected cells was found to have activity in transferring ^{3}H-UDP-GlcNAc to core 1-pNP and core 2-pNP. These results indicate that the cloned enzyme has activity as a core 1 extension β1,3-N-acetylglucosaminyltransferase.

B. Production of the MECA-79 antigen using recombinant hβ1,3GnT (SEQ ID NO: 2)

CHO cells were transfected with CD34 and either (a) no enzyme; (b) pcDNA1/hLSST-2 alone; pcDNA3.1/Zeo/mβ1,3GnT alone; or pcDNA1/hLSST-2 and pcDNA3.1/Zeo/mβ1,3GnT together using lipofectamine essentially as described above. Mock transfected and transfected cells were stained with MECA-79 antibody obtained from Pharmingen (San Diego, CA), and further incubated with goat anti-rat IgM antibodies essentially as described in Hemmerich et al., *supra*, 1994. As shown in Figure 7, positive staining with MECA-79 antibody was only observed in cells co-transfected with both hLSST-2 and mβ1,3GnT vectors, but not in cells only transfected with either enzyme alone. No other sulfotransferases examined showed MECA-79 expression when cotrasnfected into CHO cells with mβ1,3GnT. These results indicate that the human L-selectin sulfotransferase-2 and the core 1 extension enzyme β1,3GnT are sufficient to form the MECA-79 antigen when co-expressed in CHO cells.

C. Cloning and characterization of murine β1,3GnT

Several sets of primers based on the human core 1 extension β1,3GnT were used for PCR amplification of single stranded cDNA prepared from mouse small intestine using a SMART PCR cDNA synthesis kit according to the manufacturer's instructions (Clontech #K1052-1). PCR amplification was performed using the following conditions: 94°C for 2 minutes, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. Only one set of primers gave a specific amplification product. Primers A7 (5'-TTCCTGCTGCTGGTGATCAAGTCC-3'; SEQ ID NO: 13), which corresponds to human β1,3GnT nucleotides 335 to 358) and primer A3AS (5'-CAGGACCTGCTTGAGCGTGAGGTTG-3'; SEQ ID NO: 14), which corresponds to human β1,3GnT nucleotides 560 to 585, gave a product of 251 bp.

5'- and 3'-RACE were performed to isolate additional murine β1,3GnT sequence. 5'-RACE was performed using Marathon-Ready mouse testis cDNA (Clontech) using mA2AS primer 5'-ATGGAAATCCCACTGGAGAATGTCGCCGT-3' (SEQ ID NO: 15) and the AP1 primer provided by Marathon-Ready cDNA kit. 3'-RACE was performed using mA1 primer 5'-GCCTGCAAACTATGGGCGCCGCCAGAT-3' (SEQ ID NO: 16) and the SMART primer (Clontech) on mouse small intestine single stranded cDNA prepared using Clontech's SMART PCR cDNA synthesis kit as a template. The full-length cDNA was amplified based on the RACE sequence from mouse small intestine single-stranded cDNA and subcloned into pcDNA3.1/Zeo and designated pcDNA3.1/Zeo/mβ13,GnT.

EXAMPLE II

CLONING OF HUMAN L-SELECTIN LIGAND SULFOTRANSFERASE (LSST-2)

This example describes the isolation of a nucleic acid molecule encoding human L-selectin ligand sulfotransferase-2 (LSST-2), which, together with the β1,3-N-acetylglucosaminyltransferase, directs expression of the MECA-79 antigen.

Like other sulfotransferases in the same gene family (Mazany et al., Biochim. Biophys. Acta 1407:92-97 (1998)), the coding sequence for human LSST-2 was expected to reside in a single exon. Thus, human genomic DNA was used as the template for PCR-based cloning. Primers corresponding to nucleotides 891 to 910 and nucleotides 1327-1302 of mouse LSST-1 (Hiraoka et al., *supra,* 1999) were used to amplify human genomic DNA as follows. Samples were denatured for 3 minutes at 94°C, followed by 40 cycles of 1 minute at 94°C, 30 seconds at 61°C, and 45 seconds at 72°C. The amplified products were cloned into pBluescript by TA cloning. The resultant coding sequence was 79.2% identical to mouse LSST-1 at the nucleotide level.

To clone the full-length LSST-2 coding sequence, a P1 phage library of human genomic DNA (Genome System Inc.; St. Louis, MO) was PCR-amplified using primers 5'-CCGAATTCTCCCGAGAACGCACAAAG-3' (SEQ ID NO: 17) and 5'-CCCAAGCTTCTCATAGCGCACAAGCAG-3' (SEQ ID NO: 18). The PCR was carried out for 30 cycles using a 67°C annealing temperature. From the single positive clone, DNA was purified and sequenced directly. The coding

sequence present on the single exon was confirmed by reverse transcriptase (RT)-PCR using poly(A)$^+$ RNA isolated from human lymph node, as described previously (Hiraoka et al., *supra*, 1999). Three pairs of primers used in these PCR reactions correspond to 5'-TTGGCCAGAAGGGGAATAG-3' (SEQ ID NO: 19) and 5'-CCACTGAAAGAGGCTGGACTGT-3' (SEQ ID NO: 20); 5'-GGTTCTGTCTTCCTGGCGCTC-3' (SEQ ID NO: 21) and 5'-TTTGGCAGATGACCTGCATCAC-3' (SEQ ID NO: 22); and 5'-AGAACGCACAAAGGAGATCTCA-3' (SEQ ID NO: 23) and 5'-AGATGTAGGCAAGGCTCAGAAG-3' (SEQ ID NO: 24). PCR with the first two pairs of primers was performed by denaturation for 3 minutes at 94°C, followed by 35 cycles of 1 minute at 94°C, 30 seconds at 56°C, and 1 minute at 72°C. For the PCR with the third pair of primers, the annealing temperature was changed to 55°C. With the first pair of primers (SEQ ID NOS: 19 and 20), the expected characteristic fragment of 470 bp was obtained. With the second pair of primers (SEQ ID NOS: 21 and 22), the expected characteristic fragment of 617 bp was obtained. With the third pair of primers (SEQ ID NOS: 23 and 24), the expected characteristic fragment of 600 bp was obtained.

The cDNA containing full-length coding sequence of human LSST-2 was excised by *Xba*I and *Tfi*I, blunt-ended and cloned into pcDNA1.1 (Invitrogen). The resulting LSST-2 expression vector, in which the LSST-2 coding sequence is expressed under control of the CMV promoter, was designated pcDNA1.1/LSST-2.

EXAMPLE III

FUNCTIONAL ANALYSIS OF HUMAN β1,3GnT

This example describes the function of hβ1,3GnT when stably expressed in CHO cells with hLSST-2.

The following CHO cell lines were generated by stable transfection: CHO/CD34/FT7/hLSST-2; CHO/CD34/FT7/hLSST-2/C2GnT-L; CHO/CD34/FT7/hLSST-2/core 1 extension β1,3GnT; and CHO/CD34/FT7/hLSST-2/C2GnT-L/core 1 extension β1,3GnT.

The stable cell lines were established by standard procedures. Cells were selected with a combination of neomycin, hygromycin and zeocin. The expression of each gene was confirmed by immunostaining with specific antibodies against the relevant cell surface antigens.

Expression of human CD34 was confirmed by the positive staining of cells with anti-human CD34 antibody. CHO/CD34/FT7/hLSST-2 was first established. The expression of human fucosyltransferase 7 (FT7) was confirmed by the positive staining of cells with anti-sialyl Lewis x (product of FT7) antibody 2H5 as described in Kimura et al., Proc. Natl. Acad. Sci., USA 96:4530-4535 (1997). Expression of hLSST-2 was confirmed by transient transfection of β1,3GnT-A (core 1 extension β1,3GnT) and cells were stained with MECA-79 as described above. For the confirmation of C2GnT expression in the CHO/CD34/FT7/hLSST/C2GnT-L cell line, the NCC-ST-439 antibody against sialyl Lewis x core 2 structure was used essentially as described in Kumamoto

et al., Biochim. Biophys. Res. Comm. 247:514-517 (1998). For the confirmation of core 1 extension β1,3GnT expression in the CHO/CD34/FT7/hLSST/core 1 extension β1,3GnT cell line, MECA-79 antibody staining was performed as described above.

Cells were grown as a monolayer on tissue culture flasks, and mouse lymphocytes were allowed to flow over the monolayer under different shear forces essentially as described in Fuhlbrigge et al., J. Cell Biol. 135:837-48 (1996). The number of lymphocytes which rolled on the cell monolayer were monitored by video camera and counted. As shown in Figure 8, CHO cells expressing either the core 2 extension enzyme, C2GnT-L (open square) or the human core 1 extension enzyme, β1,3GnT (filled square) rolled more than cells only expressing fucosyltransferase VII (FT7; open circle). Furthermore, rolling was significantly enhanced when lymphocytes rolled on cells expressing both the core 2 extension enzyme, C2GnT-L, and human β1,3GnT (filled circle). These results indicate that both core 1 and core 2 extended sulfo sialyl Lewis X determinants play a role in lymphocyte homing.

EXAMPLE IV

DEFINITION OF THE MINIMUM EPITOPE OF THE MECA-79 ANTIGEN

This example describes the use of ELISA analysis to define the minimum epitope of the MECA-79 antigen.

A. ELISA Assays with anti-MECA-79 Antibody

The stable CHO transfectants described in Example III were grown on 10 cm plates and transiently transfected with soluble form of human CD34 (pcDNA3.1/HSH vector) using LipofectAmine PLUS (GibcoBRL). One day after transfection, the culture media was replaced with 10 ml of OptiMEM reduced-serum medium. After culturing for an additional two days, the culture media was collected. Cell debris was removed by centrifugation, and the media was concentrated 10-fold by Centriprep 10 concentrators.

The concentrated media was diluted 100-fold in Tris-buffered saline (TBS; 50 mM Tris, 150 mM NaCl, pH 7.5). The wells of 96-well polystyrene microtiter plates (Nunc, F96 Maxisorp Cat. # 442404) were coated overnight with 100 µl of diluted media at 4°C. The plates were washed three times with TBS and were blocked with 250 µl of 5% BSA (in TBS) at room temperature for at least two hours (or 4°C overnight). The wells were washed three times with washing buffer (TBS containing 0.1% Tween 20). Two-fold serially diluted MECA-79 antibody at a concentration of 1:200 to 1:12,800 (Pharmingen) was prepared in dilution buffer (5% BSA in washing buffer). Fifty µl of diluted antibodies were added to each well and incubated at room temperature for one hour. After washing three times with washing buffer, 50 ml of anti-rat IgM-alkaline phosphatase conjugate (1:500 in dilution buffer) was added to each well and allowed to incubate at room temperature for one hour. Following washing three times with washing buffer, the wells were washed twice with deionized water. Alkaline

phosphatase substrate p-nitro-phenylphosphate (1 mg/ml) was freshly prepared in bicarbonate buffer (0.1 M NaHCO3, pH 9.6) containing 0.5 mM $MgCl_2$. Fifty microliter of this substrate solution was added to each well and allowed to incubate at 37°C. The optical density at 405 nm of each well was recorded using Spectra MAX Plus microtiter plate reader (Molecular Device Corp.). Positive readings were observed from the media of CHO cells harboring both hLSST-2 and core 1 extension β1,3GnT and CHO/CD34/FT7/hLSST-2/core 1 extension β1,3GnT/core2GnT).

B. Inhibition of MECA-79 Antibody Binding by Synthetic Oligosaccharides

Synthetic oligosaccharides were mixed at the indicated concentrations with MECA-79 antibody (at a final dilution of 1:10,000). The mixtures were incubated at room temperature for one hour before addition to wells precoated with transfected media from CHO/CD34/FT7/LSST/core 1 extension β1,3GnT cells as above. Antibody binding was assayed as described above.

The results shown in Figure 9 indicate that only the 6-S-extended core 1 structure (Galβ1→4(SO_3→6) GlcNAcβ1→3Galβ1→3GalNAc) was active in inhibiting binding of anti-MECA-79 antibody. Thus, these results define the minimum epitope of MECA-79 as Galβ1→4(SO_3→6) GlcNAcβ1→3Galβ1→3GalNAc.

To further confirm and refine the requirements for the MECA-79 reactivity, oligosaccharides derived from the extended core 1 glycan were chemically synthesized and examined for their ability to inhibit MECA-79

antibody binding to MECA-79-reactive CD34 chimeric proteins. MECA-79 antibody binding was efficiently inhibited by a 6-sulfo extended core 1 oligosaccharide, Galβ→4(sulfo→6)GlcNAcβ1→3Galβ1→3GalNAcα1→octyl, and its sialylated or sialylated, fucosylated forms, NeuNAcα2→3Galβ1→4[Fucα1→3(sulfo→6)]GlcNAcβ1→3Galβ1→3GalNAcα1→octyl (see Figure 9). The 6-sulfo group was absolutely required, since non-sulfated, extended core 1 oligosaccharide did not inhibit MECA-79 antibody binding (Figure 9). In addition, the terminal galactose residue in the *N*-acetyllactosaminyl core 1 was part of the epitope, since the agalacto form required more than a 10 fold increase concentration to achieve equivalent inhibition (Figure 9). An absolute requirement for core 1 structure was also demonstrated, since sulfated *N*-acetyllactosamine lacking a core 1 structure did not show detectable inhibition. These results are consistent with previous studies showing that sialic acid and fucose are not integral parts of the MECA-79 epitope (Hemmerich et al., *supra,* 1994; Maly et al., *supra,* 1996). The results also are consistent with previous findings that the MECA-79 antibody can inhibit lymphocyte homing without prior removal of sialic acid or fucose (Streeter et al., *supra,* 1988; Clark et al., J. Biol. Chem. 140:721-731 (1998)).

These results demonstrate that the minimum epitope of MECA-79 is the sulfated, extended core1 structure Galβ1→4(sulfo→6)GlcNAcβ1→3Galβ1→3GalNAcα1→R, and that sialylated and sialylated, fucosylated forms of this structure (6-sulfo sLe^x in extended core 1 forms) retain MECA-79 reactivity.

EXAMPLE V

MURINE INTESTINAL GlcNAc 6-SULFOTRANSFERASE

This example describes the cloning and characterization of the murine intestinal GlcNAc 6-sulfotransferase.

The coding sequence of mouse LSST-1 (Hiraoka et al., Immunity 11:79-89 (1999)) was used as probe to search dbEST using tblstx program. One unknown query gene (AI115260) was found to have 53.8% identity with the coding regions of mouse LSST-1. AI115260 is a sequence isolated from mouse embryo cDNA. Sequence analysis of this cDNA, obtained from Genome Systems (St. Louis, MS), revealed that this cDNA encodes a protein of 396 amino acids, designated intestinal GlcNAc 6-sulfotransferase. The cDNA insert was digested with EcoRI and XbaI and cloned into the corresponding sites of pcDNA3.1 (Invitrogen) to produce the expression vector pcDNA3-I-GlcNAc6ST.

Lec2 cells, which are defective in Golgi sialylation due to a CMP-sialic acid transporter defect, were doubly transfected with pcDNA3-I-GlcNAc6ST and pcDNA3.1/hβ1,3GnT-A. Because of the absence of sialic acid in Lec2 cells, core 1 extension occurs with the competition of sialylation and, therefore, more core 1 extended structure is formed by the core 1 extension enzyme β1,3GnT. Under these conditions, the MECA-79 antigen was produced in the doubly transfected Lec2 cells. Similar production of MECA-79 antigen was observed when Lec2 cells were doubly transfected with mLSST-1 and hβ1,3GnT (SEQ ID NO: 2). These results

indicate that, under certain conditions, mLSST-1 or I-GLCNAc6ST can form the MECA-79 antigen.

All journal article, reference, and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. A method of treating or preventing an L-selectin-mediated condition in a subject, comprising reducing the expression or activity of a β1,3GnT that directs expression of a MECA-79 antigen.

2. The method of claim 1, comprising administering to said subject an oligosaccharide L-selectin antagonist that inhibits the binding of L-selectin to a MECA-79 antigen.

3. The method of claim 2, wherein said L-selectin antagonist comprises the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc.

4. The method of claim 3, wherein said L-selectin antagonist comprises NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1.

5. The method of claim 3, wherein said L-selectin antagonist comprises two or more of the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc.

6. The method of claim 4, wherein said L-selectin antagonist comprises two or more of the oligosaccharide NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1.

7. The method of claim 1, comprising administering to said subject inhibitory antibody material that specifically binds β1,3GnT.

8. The method of claim 1, comprising administering to said subject a β1,3GnT antisense nucleic acid molecule.

9. The method of claim 8, wherein said antisense nucleic acid molecule has at least 20 nucleotides complementary to SEQ ID NO: 1.

10. The method of claim 9, wherein said antisense nucleic acid molecule has at least 20 nucleotides complementary to SEQ ID NO: 3.

11. The method of claim 1, further comprising reducing the expression or activity of L-selectin sulfotransferase-2 (LSST-2) in said subject.

12. An isolated L-selectin antagonist, comprising an extended core 1 structure comprising the oligosaccharide Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc.

13. The isolated L-selectin antagonist of claim 12, comprising the oligosaccharide NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1.

14. The isolated L-selectin antagonist of claim 12, comprising two or more of the oligosaccharides Galβ1→4(SO_3→6)GlcNAcβ1→3Galβ1→3GalNAc.

15. The isolated L-selectin antagonist of claim 13, comprising two or more of the oligosaccharides NeuNAcα2→3Galβ1→4[sulfo→6(Fucα1→3)GlcNAc]β1→3Galβ1→3GalNAcα1.

16. An isolated nucleic acid molecule, comprising a nucleic acid sequence encoding a L-selectin ligand sulfotransferase (LSST-2) or active fragment thereof, wherein said LSST-2 or active fragment thereof directs expression of a MECA-79 antigen in Chinese hamster ovary (CHO) cells.

17. The isolated nucleic acid molecule of claim 16, wherein said LSST-2 has substantially the amino acid sequence of SEQ ID NO: 6.

18. The isolated nucleic acid molecule of claim 17, comprising a nucleic acid sequence encoding SEQ ID NO: 6.

19. The isolated nucleic acid molecule of claim 18, comprising SEQ ID NO: 5.

20. An isolated polypeptide, comprising an amino acid sequence encoding a LSST-2 or active fragment thereof, wherein said LSST-2 or active fragment thereof directs expression of a MECA-79 antigen in CHO cells.

21. The isolated polypeptide of claim 20, wherein said LSST-2 has substantially the amino acid sequence of SEQ ID NO: 6.

22. The isolated polypeptide of claim 21, wherein said LSST-2 has the amino acid sequence SEQ ID NO: 6.

23. An isolated nucleic acid molecule, comprising a nucleic acid sequence encoding substantially the amino acid sequence of intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) or an active fragment thereof.

24. The isolated nucleic acid molecule of claim 23, wherein said I-GlcNAc6ST has substantially the amino acid sequence of SEQ ID NO: 8.

25. The isolated nucleic acid molecule of claim 24, comprising a nucleic acid sequence encoding SEQ ID NO: 8.

26. The isolated nucleic acid molecule of claim 25, comprising SEQ ID NO: 7.

27. An isolated polypeptide, comprising an amino acid sequence encoding substantially the amino acid sequence of intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) or an active fragment thereof.

28. The isolated polypeptide of claim 27, wherein said I-GlcNAc6ST has substantially the amino acid sequence of SEQ ID NO: 8.

29. The isolated polypeptide of claim 28, wherein said I-GlcNAc6ST has the amino acid sequence SEQ ID NO: 8.

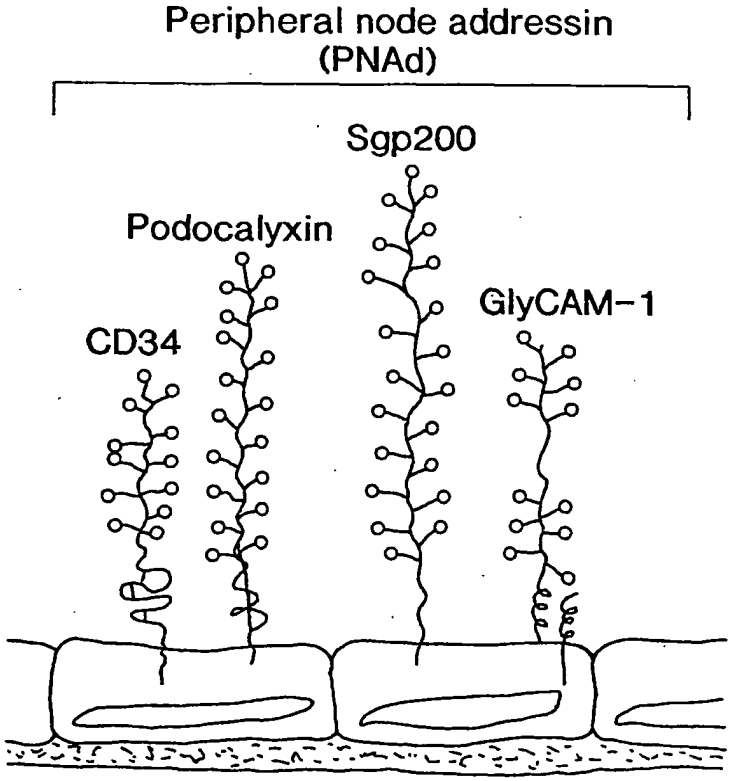
Peripheral node addressin
(PNAd)
Sgp200
Podocalyxin
GlyCAM-1
CD34

FIG. 1

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CTGGCTGGCCAGGATGAAGTATCTCCGGCACCGGCGGCCCAATGCCACCCTCATTCTGGC   60
          M  K  Y  L  R  H  R  R  P  N  A  T  L  I  L  A      16

CATCGGCGCTTTCACCCTCCTCCTCTTCAGTCTGCTAGTGTCACCACCCACCTGCAAGGT  120
 I  G  A  F  T  L  L  L  F  S  L  L  V  S  P  P  T  C  K  V   36

CCAGGAGCAGCCACCGGCGATCCCCGAGGCCCTGGCCTGGCCCACTCCACCCACCCGCCC  180
 Q  E  Q  P  P  A  I  P  E  A  L  A  W  P  T  P  P  T  R  P   56

AGCCCCGGCCCCGTGCCATGCCAACACCTCTATGGTCACCCACCCGGACTTCGCCACGCA  240
 A  P  A  P  C  H  A  N  T  S  M  V  T  H  P  D  F  A  T  Q   76

GCCGCAGCACGTTCAGAACTTCCTCCTGTACAGACACTGCCGCCACTTTCCCCTGCTGCA  300
 P  Q  H  V  Q  N  F  L  L  Y  R  H  C  R  H  F  P  L  L  Q   96

GGACGTGCCCCCCTCTAAGTGCGCGCAGCCGGTCTTCCTGCTGCTGGTGATCAAGTCCTC  360
 D  V  P  P  S  K  C  A  Q  P  V  F  L  L  L  V  I  K  S  S  116

CCCTAGCAACTATGTGCGCCGCGAGCTGCTGCGGCGCACGTGGGGCCGCGAGCGCAAGGT  420
 P  S  N  Y  V  R  R  E  L  L  R  R  T  W  G  R  E  R  K  V  136

ACGGGGTTTGCAGCTGCGCCTCCTCTTCCTGGTGGGCACAGCCTCCAACCCGCACGAGGC  480
 R  G  L  Q  L  R  L  L  F  L  V  G  T  A  S  N  P  H  E  A  156

CCGCAAGGTCAACCGGCTGCTGGAGCTGGAGGCACAGACTCACGGAGACATCCTGCAGTG  540
 R  K  V  N  R  L  L  E  L  E  A  Q  T  H  G  D  I  L  Q  W  156

GGACTTCCACGACTCCTTCTTCAACCTCACGCTCAAGCAGGTCCTGTTCTTACAGTGGCA  600
 D  F  H  D  S  F  F  N  L  T  L  K  Q  V  L  F  L  Q  W  Q  176

GGAGACAAGGTGCGCCAACGCCAGCTTCGTGCTCAACGGGGATGATGACGTCTTTGCACA  660
 E  T  R  C  A  N  A  S  F  V  L  N  G  D  D  D  V  F  A  H  196

CACAGACAACATGGTCTTCTACCTGCAGGACCATGACCCTGGCCGCCACCTCTTCGTGGG  720
 T  D  N  M  V  F  Y  L  Q  D  H  D  P  G  R  H  L  F  V  G  216

GCAACTGATCCAAAACGTGGGCCCCATCCGGGCTTTTTGGAGCAAGTACTATGTGCCAGA  780
 Q  L  I  Q  N  V  G  P  I  R  A  F  W  S  K  Y  Y  V  P  E  236

GGTGGTGACTCAGAATGAGCGGTACCCACCCTATTGTGGGGGTGGTGGCTTCTTGCTGTC  840
 V  V  T  Q  N  E  R  Y  P  P  Y  C  G  G  G  G  F  L  L  S  256

CCGCTTCACGGCCGCTGCCCTGCGCCGTGCTGCCCATGTCTTGGACATCTTCCCCATTGA  900
 R  F  T  A  A  A  L  R  R  A  A  H  V  L  D  I  F  P  I  D  276

TGATGTCTTCCTGGGTATGTGTCTGGAGCTTGAGGGACTGAAGCCTGCCTCCCACAGCGG  960
 D  V  F  L  G  M  C  L  E  L  E  G  L  K  P  A  S  H  S  G  296

CATCCGCACGTCTGGCGTGCGGGCTCCATCGCAACACCTGTCCTCCTTTGACCCCTGCTT 1020
 I  R  T  S  G  V  R  A  P  S  Q  H  L  S  S  F  D  P  C  F  316

CTACCGAGACCTGCTGCTGGTGCACCGCTTCCTACCTTATGAGATGCTGCTCATGTGGGA 1080
 Y  R  D  L  L  L  V  H  R  F  L  P  Y  E  M  L  L  M  W  D  336
```

FIG. 2

```
TGCGCTGAACCAGCCCAACCTCACCTGCGGCAATCAGACACAGATCTACTGAGTCAGCAT 1140
 A  L  N  Q  P  N  L  T  C  G  N  Q  T  Q  I  Y  *           352

CAGGCATCCGCACGTCTGGCGTGCGGGCTCCATCGCAACACCTGTCCTCCTTTGACCCCT 1200
GCTTCTAC                                                      1210
```

FIG. 2 CONT.

```
AGGCTCCGCCCCCACGCCATGCGGCTGCCAAGGCAGAGCCCCTACGAGATCCTCCTCCTG      60
                  M  R  L  P  R  Q  S  P  Y  E  I  L  L  L        14

GTCTTGGTCGCCTTGCTGGTGCTGCTGCTGCTCCTGACCAGCAAGTCACCGCCCAGCTGC     120
V  L  V  A  L  L  V  L  L  L  L  L  T  S  K  S  P  P  S  C      34

TCCGCCCCTGAGAGGTCCAAGGAGCCTGAAGACAACCCCGGGTGGGCCACGGGCCACCCC     180
S  A  P  E  R  S  K  E  P  E  D  N  P  G  W  A  T  G  H  P      54

GCCCGGTGCCGAGCTAATCTATCCGTGTCCTCGCACCCCGACTTCGCGGGGCTGCCCTTG     240
A  R  C  R  A  N  L  S  V  S  S  H  P  D  F  A  G  L  P  L      74

CACGTGCGCGACTTCTTGTTCTACCGCCACTGCCGCGACTTCCCAGTGCTCCGAGAGCCG     300
H  V  R  D  F  L  F  Y  R  H  C  R  D  F  P  V  L  R  E  P      94

CGGGTTACCAAGTGCGCGGAGCCCGTGTTCCTGCTGCTCGCCATCAAGTCCTCGCCTGCA     360
R  V  T  K  C  A  E  P  V  F  L  L  L  A  I  K  S  S  P  A     114

AACTATGGGCGCCGCCAGATGCTGCGCACGACGTGGGCGCGCGAGAGACGGGTGCGTGGG     420
N  Y  G  R  R  Q  M  L  R  T  T  W  A  R  E  R  R  V  R  G     134

GCGCCACTGCGCCGCCTCTTCCTTGTGGGCTCAGACCGCGACCCACAACAAGCACGCAAA     480
A  P  L  R  R  L  F  L  V  G  S  D  R  D  P  Q  Q  A  R  K     154

TACAACCGACTGCTGGAGCTGGAAGCGCAGAAATACGGCGACATTCTCCAGTGGGATTTC     540
Y  N  R  L  L  E  L  E  A  Q  K  Y  G  D  I  L  Q  W  D  F     174

CATGACTCCTTCTTTAACCTGACGCTTAAGCAGGTCCTTTTCCTGGAGTGGCAGCTAACC     600
H  D  S  F  F  N  L  T  L  K  Q  V  L  F  L  E  W  Q  L  T     194

TACTGTACCAACGCCAGCTTCGTGCTCAATGGGGACGACGATGTGTTCGCACACACGGAC     660
Y  C  T  N  A  S  F  V  L  N  G  D  D  D  V  F  A  H  T  D     214

AACATGGTCACCTACCTGCAGGACCACGACCCGGACCAACACCTCTTCGTGGGGCACCTG     720
N  M  V  T  Y  L  Q  D  H  D  P  D  Q  H  L  F  V  G  H  L     234

ATCCAGAACGTGGGTCCCATCCGGGTGCCCTGGAGCAAGTACTTCATCCCCGCTCTGGTG     780
I  Q  N  V  G  P  I  R  V  P  W  S  K  Y  F  I  P  A  L  V     254

ATGGCGGAGGACAGATACCCGCCCTACTGTGGTGGCGGCGGCTTCCTGCTGTCGCGTTTT     840
M  A  E  D  R  Y  P  P  Y  C  G  G  G  G  F  L  L  S  R  F     274

ACCGTGGCCGCCCTACGTCGCGCCGCGCGCGTCCTCCCCATGTTCCCAATCGACGACGTG     900
T  V  A  A  L  R  R  A  A  R  V  L  P  M  F  P  I  D  D  V     294

TTCCTGGGCATGTGTCTGCAGCAGCAGGGTCTGGCTCCCGGGACGCACAGCGGAGTGCGC     960
F  L  G  M  C  L  Q  Q  Q  G  L  A  P  G  T  H  S  G  V  R     314

ACTGCGGGGGTTTTCCCCCCTAGCCCACGTGTGTCATCCTTCGACCCCTGCTTCTACCGC    1020
T  A  G  V  F  P  P  S  P  R  V  S  S  F  D  P  C  F  Y  R     334

GACCTGCTCCTCGTGCACCGCTTCCTGCCCTTCGAGATGCTGCTGATGTGGGATGCGCTG    1080
D  L  L  L  V  H  R  F  L  P  F  E  M  L  L  M  W  D  A  L     354
```

FIG. 3

```
AACCAGCCCCAGCTCCTCTGCGGCAGGCAGAGCCCCGCCTACTGAGAGGTTTGGGGGAGT   1140
N  Q  P  Q  L  L  C  G  R  Q  S  P  A  Y  *                     368

TGACATCCCCTAGCTCATGTCCTGCCTCATCCACGTGCAAAGGGCTGGCTTCAAGGAGAA   1200
GTTCAAAGTGAGGGGCAGAAAGGTGGGTCTGAGGAGTTCATAGGGCAAACTCCTAAGTAC   1260
GCTTGGAAACCCTCTTGGTACTGTTCACAGCAGGAACTCTGAGTCTAGCCAACTCTGAGT   1320
GGCTCTAAGTGGCCGCT                                              1337
```

FIG. 3 CONT.

```
1   TTGGCCAGAAGGGGAATAGAAGGCAAACAATAAAACAGCAGCCCAACTCCACCCTTTCTG  60

61  TTTGTTCCTTAAAGGTCTTCCACTTCAGCACAATGCTACTGCCTAAAAAAATGAAGCTCC  120
                                                    M  K  L  L

121 TGCTGTTTCTGGTTTCCCAGATGGCCATCTTGGCTCTATTCTTCCACATGTACAGCCACA  180
      L  F  L  V  S  Q  M  A  I  L  A  L  F  F  H  M  Y  S  H  N

181 ACATCAGCTCCCTGTCTATGAAGGCACAGCCCGAGCGCATGCACGTGCTGGTTCTGTCTT  240
      I  S  S  L  S  M  K  A  Q  P  E  R  M  H  V  L  V  L  S  S

241 CCTGGCGCTCTGGCTCTTCTTTTGTGGGGCAGCTTTTTGGGCAGCACCCAGATGTTTTCT  300
      W  R  S  G  S  S  F  V  G  Q  L  F  G  Q  H  P  D  V  F  Y

301 ACCTGATGGAGCCCGCCTGGCACGTGTGGATGACCTTCAAGCAGAGCACCGCCTGGATGC  360
      L  M  E  P  A  W  H  V  W  M  T  F  K  Q  S  T  A  W  M  L

361 TGCACATGGCTGTGCGGGATCTGATACGGGCCGTCTTCTTGTGCGACATGAGCGTCTTTG  420
      H  M  A  V  R  D  L  I  R  A  V  F  L  C  D  M  S  V  F  D

421 ATGCCTACATGGAACCTGGTCCCCGGAGACAGTCCAGCCTCTTTCAGTGGGAGAACAGCC  480
      A  Y  M  E  P  G  P  R  R  Q  S  S  L  F  Q  W  E  N  S  R

481 GGGCCCTGTGTTCTGCACCTGCCTGTGACATCATCCCACAAGATGAAATCATCCCCCGGG  540
      A  L  C  S  A  P  A  C  D  I  I  P  Q  D  E  I  I  P  R  A

541 CTCACTGCAGGCTCCTGTGCAGTCAACAGCCCTTTGAGGTGGTGGAGAAGGCCTGCCGCT  600
      H  C  R  L  L  C  S  Q  Q  P  F  E  V  V  E  K  A  C  R  S

601 CCTACAGCCACGTGGTGCTCAAGGAGGTGCGCTTCTTCAACCTGCAGTCCCTCTACCCGC  660
      Y  S  H  V  V  L  K  E  V  R  F  F  N  L  Q  S  L  Y  P  L

661 TGCTGAAAGACCCCTCCCTCAACCTGCATATCGTGCACCTGGTCCGGGACCCCCGGGCCG  720
      L  K  D  P  S  L  N  L  H  I  V  H  L  V  R  D  P  R  A  V

721 TGTTCCGTTCCCGAGAACGCACAAAGGGAGATCTCATGATTGACAGTCGCATTGTGATGG  780
      F  R  S  R  E  R  T  K  G  D  L  M  I  D  S  R  I  V  M  G

781 GGCAGCATGAGCAAAAACTCAAGAAGGAGGACCAACCCTACTATGTGATGCAGGTCATCT  840
      Q  H  E  Q  K  L  K  K  E  D  Q  P  Y  Y  V  M  Q  V  I  C

841 GCCAAAGCCAGCTGGAGATCTACAAGACCATCCAGTCCTTGCCCAAGGCCCTGCAGGAAC  900
      Q  S  Q  L  E  I  Y  K  T  I  Q  S  L  P  K  A  L  Q  E  R
```

FIG. 4

```
901  GCTACCTGCTTGTGCGCTATGAGGACCTGGCTCGAGCCCCTGTGGCCCAGACTTCCCGAA  960
       Y  L  L  V  R  Y  E  D  L  A  R  A  P  V  A  Q  T  S  R  M

961  TGTATGAATTCGTGGGATTGGAATTCTTGCCCCATCTTCAGACCTGGGTGCATAACATCA  1020
       Y  E  F  V  G  L  E  F  L  P  H  L  Q  T  W  V  H  N  I  T

1021 CCCGAGGCAAGGGCATGGGTGACCACGCTTTCCACACAAATGCCAGGGATGCCCTTAATG  1080
       R  G  K  G  M  G  D  H  A  F  H  T  N  A  R  D  A  L  N  V

1081 TCTCCCAGGCTTGGCGCTGGTCTTTGCCCTATGAAAAGGTTTCTCGACTTCAGAAAGCCT  1140
       S  Q  A  W  R  W  S  L  P  Y  E  K  V  S  R  L  Q  K  A  C

1141 GTGGCGATGCCATGAATTTGCTGGGCTACCGCCACGTCAGATCTGAACAAGAACAGAGAA  1200
       G  D  A  M  N  L  L  G  Y  R  H  V  R  S  E  Q  E  Q  R  N

1201 ACCTGTTGCTGGATCTTCTGTCTACCTGGACTGTCCCTGAGCAAATCCACTAAGAGGGTT  1260
       L  L  L  D  L  L  S  T  W  T  V  P  E  Q  I  H  *

1261 GAGAAGGCTTTGCTGCCACCTGGTGTCAGCCTCAGTCACTTTCTCTGAATGCTTCTGAGC  1320

1321 CTTGCCTACATCT  1333
```

FIG. 4 CONT.

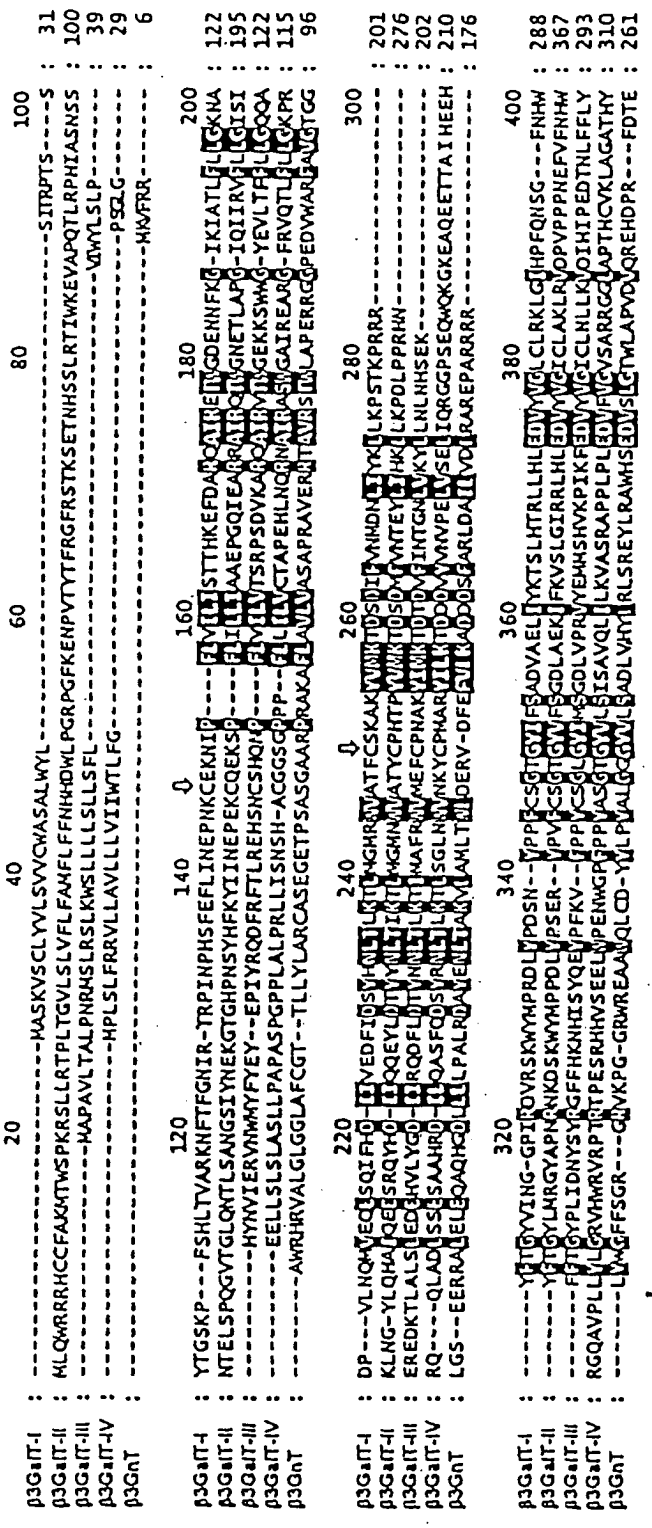

FIG. 5

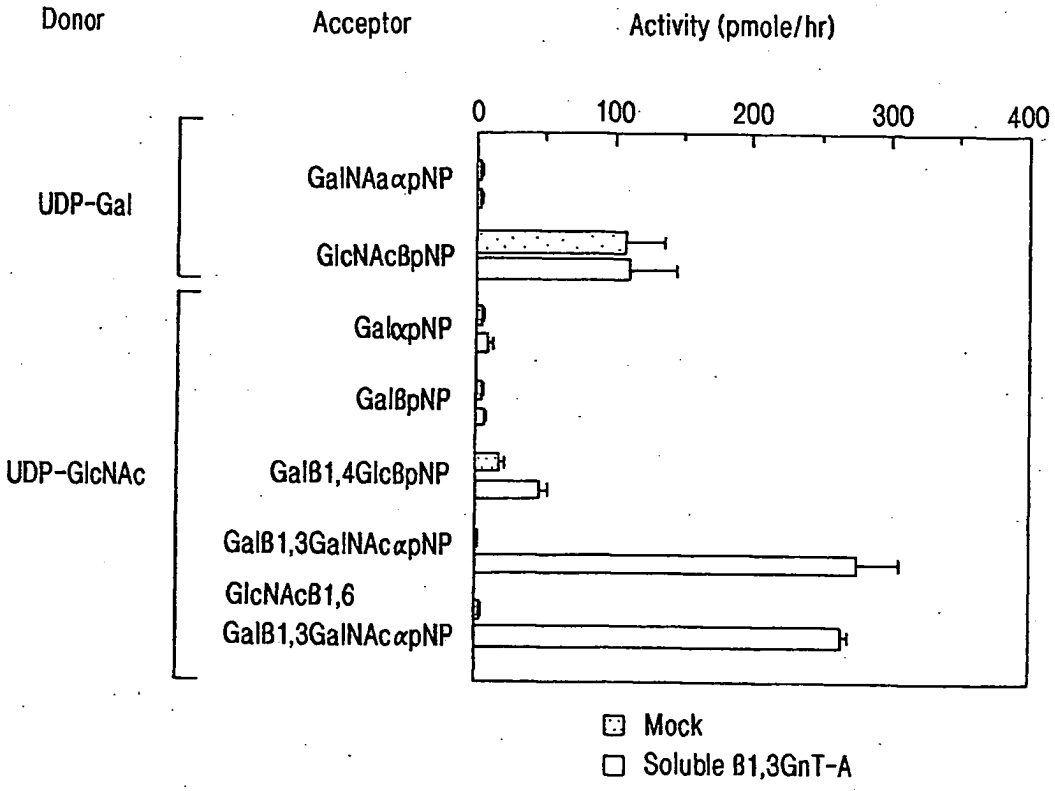
Donor
Acceptor
Activity (pmole/hr)
0
100
200
300
400
UDP-Gal
UDP-GlcNAc
GalNAcαpNP
GlcNAcβpNP
GalαpNP
GalβpNP
Galβ1,4GlcβpNP
Galβ1,3GalNAcαpNP
GlcNAcβ1,6
Galβ1,3GalNAcαpNP
Mock
Soluble β1,3GnT-A

FIG. 6

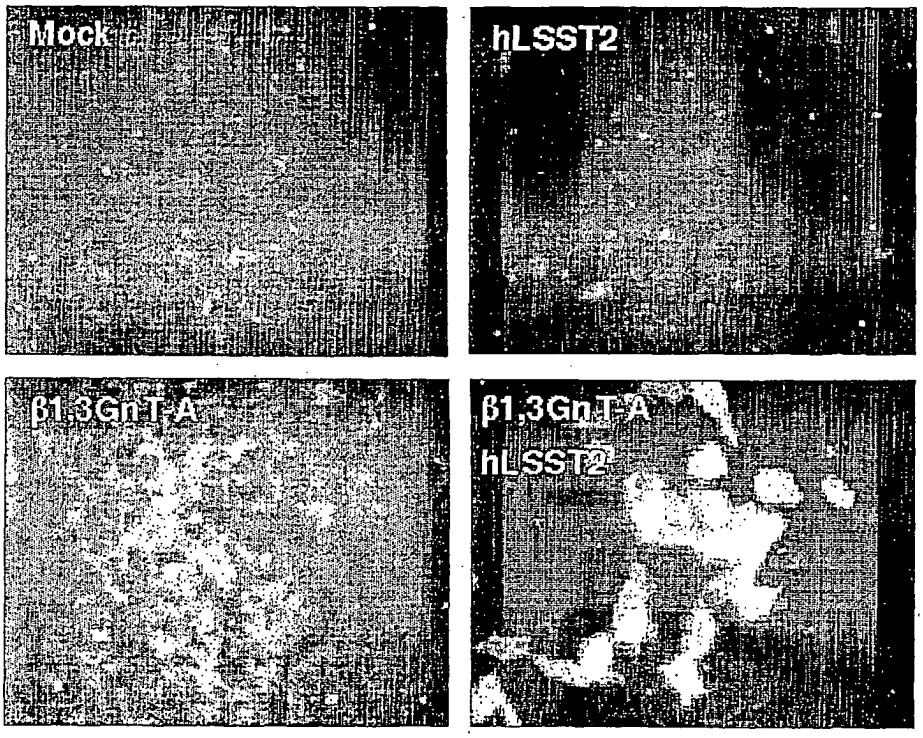

FIG. 7

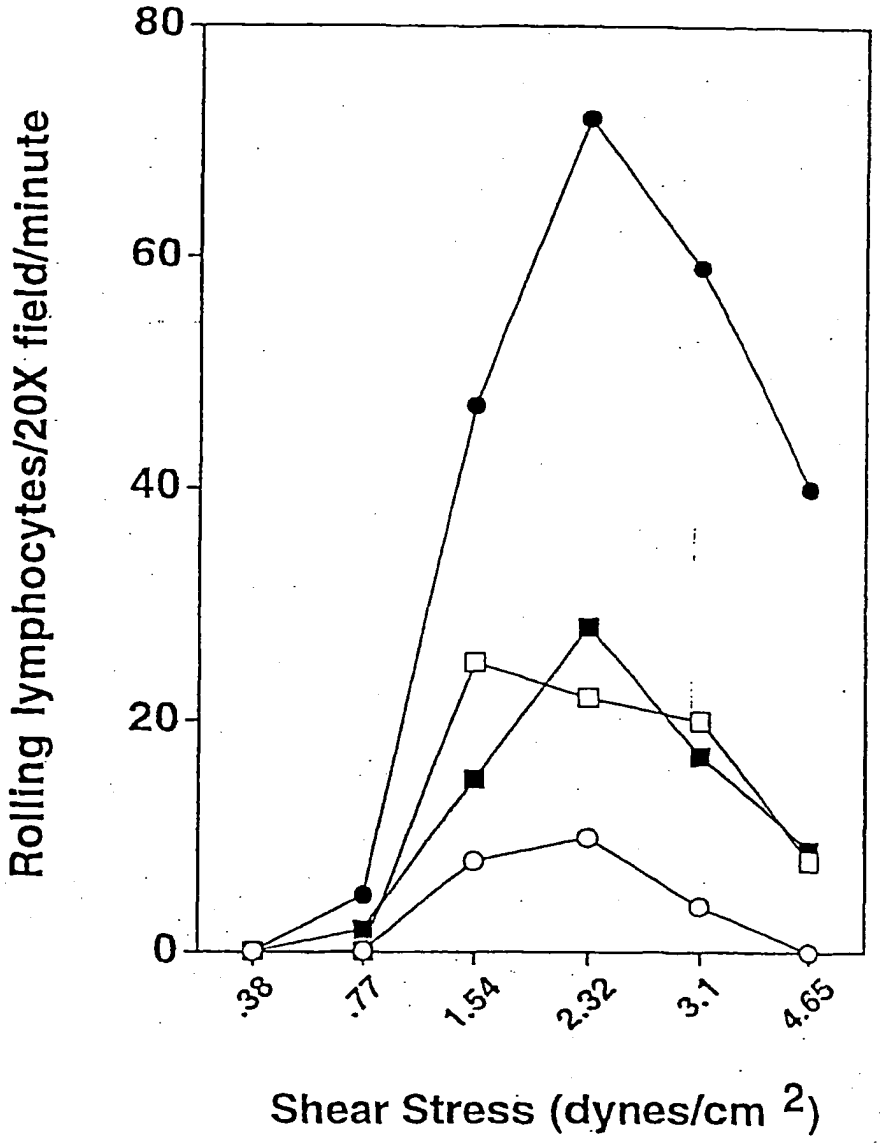
80
60
40
20
0
Rolling lymphocytes/20X field/minute
.38
.77
1.54
2.32
3.1
4.65
Shear Stress (dynes/cm 2)

FIG. 8

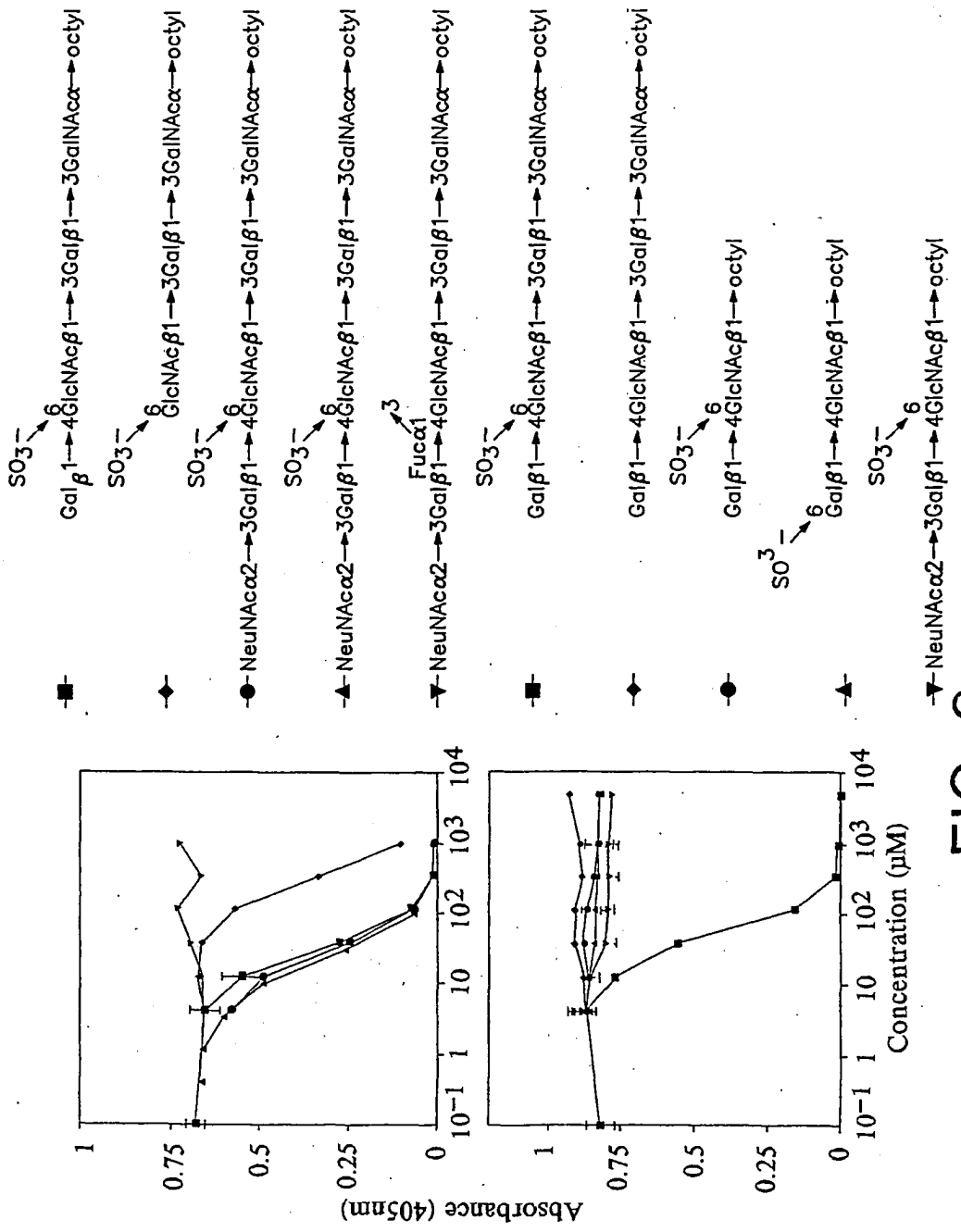
SO3−→6 Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα→octyl
SO3−→6 GlcNAcβ1→3Galβ1→3GalNAcα→octyl
SO3−→6 NeuNAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα→octyl
SO3−→6 NeuNAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα→octyl
3 Fucα1
NeuNAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα→octyl
SO3−→6 Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα→octyl
Galβ1→4GlcNAcβ1→3Galβ1→3GalNAcα→octyl
SO3−→6 Galβ1→4GlcNAcβ1→octyl
SO3−→6 Galβ1→4GlcNAcβ1→octyl
SO3−→6 NeuNAcα2→3Galβ1→4GlcNAcβ1→octyl
Absorbance (405nm)
Concentration (μM)
0
0.25
0.5
0.75
1
10−1
1
10
102
103
104

FIG. 9

```
TGAGCGGCTCTTTGTGTGCGCCCTGGGTGCGCAGCGCAGAAGCGCAGCGGGCAGCGCAGG  60

CCCTAGCCAGAGGTATGCGGCTACCCCGTTTCTCCAGCACTGTCATGCTTTCGCTCCTGA 120
              M  R  L  P  R  F  S  S  T  V  M  L  S  L  L  M

TGGTACAGACTGGCATCCTGGTCTTCCTGGTCTCCCGGCAAGTGCCATCGTCCCCAGCAG 180
  V  Q  T  G  I  L  V  F  L  V  S  R  Q  V  P  S  S  P  A  G

GCCTTGGGGAGCGTGTGCACGTGCTGGTACTGTCCTCGTGGCGCTCGGGCTCGTCCTTCG 240
  L  G  E  R  V  H  V  L  V  L  S  S  W  R  S  G  S  S  F  V

TGGGCCAGCTCTTCAGCCAACACCCCGATGTCTTCTACCTGATGGAGCCGGCTTGGCACG 300
  G  Q  L  F  S  Q  H  P  D  V  F  Y  L  M  E  P  A  W  H  V

TCTGGGATACGTTGTCGCAGGGCAGTGCCCCCGCACTCCACATGGCCGTGCGTGACCTGA 360
  W  D  T  L  S  Q  G  S  A  P  A  L  H  M  A  V  R  D  L  I

TCCGCTCAGTGTTCCTATGCGACATGGACGTATTTGATGCCTACCTGCCCTGGCGCCGCA 420
  R  S  V  F  L  C  D  M  D  V  F  D  A  Y  L  P  W  R  R  N

ACATCTCGGATCTCTTCCAGTGGGCGGTGAGCCGCGCATTGTGCTCACCTCCGGTCTGCG 480
  I  S  D  L  F  Q  W  A  V  S  R  A  L  C  S  P  P  V  C  E

AAGCCTTCGCTCGTGGCAACATCAGCAGCGAGGAGGTGTGTAAGCCTCTGTGCGCAACGC 540
  A  F  A  R  G  N  I  S  S  E  E  V  C  K  P  L  C  A  T  R

GGCCCTTCGGCCTGGCTCAGGAAGCCTGCAGCTCCTATAGTCACGTCGTGCTCAAGGAGG 600
  P  F  G  L  A  Q  E  A  C  S  S  Y  S  H  V  V  L  K  E  V

TGCGCTTCTTTAACCTACAGGTGCTCTACCCGCTGCTCAGCGACCCTGCGCTCAACCTGC 660
  R  F  F  N  L  Q  V  L  Y  P  L  L  S  D  P  A  L  N  L  R

GCATCGTGCACCTAGTGCGCGACCCGCGGGCCGTGCTGCGCTCCCGAGAGCAGACAGCCA 720
  I  V  H  L  V  R  D  P  R  A  V  L  R  S  R  E  Q  T  A  K

AGGCGCTGGCACGGGACAATGGCATCGTCCTGGGTACCAACGGCACGTGGGTGGAGGCGG 780
  A  L  A  R  D  N  G  I  V  L  G  T  N  G  T  W  V  E  A  D

ACCCCCGGCTGCGCGTGGTCAACGAGGTATGCCGCAGCCATGTGCGCATCGCAGAGGCAG 840
  P  R  L  R  V  V  N  E  V  C  R  S  H  V  R  I  A  E  A  A

CCTTGCACAAGCCGCCGCCCTTCTTGCAAGATCGCTACCGCCTGGTGCGCTACGAGGATC 900
  L  H  K  P  P  P  F  L  Q  D  R  Y  R  L  V  R  Y  E  D  L

TGGCCCGGGACCCACTCACCGTAATCCGTGAACTCTATGCCTTCACCGGCCTGGGTCTCA 960
  A  R  D  P  L  T  V  I  R  E  L  Y  A  F  T  G  L  G  L  T
```

FIG. 10

```
CGCCGCAGCTCCAGACTTGGATCCACAATATCACGCATGGTTCAGGGCCAGGCGCGCGCC  1020
  P  Q  L  Q  T  W  I  H  N  I  T  H  G  S  G  P  G  A  R  R

GTGAAGCCTTCAAGACCACATCCAGGGATGCGCTCAGTGTATCCCAGGCCTGGCGCCACA  1080
  E  A  F  K  T  T  S  R  D  A  L  S  V  S  Q  A  W  R  H  T

CGCTGCCCTTTGCCAAGATTCGCCGGGTCCAGGAACTGTGCGGGGGTGCACTGCAGCTGC  1140
  L  P  F  A  K  I  R  R  V  Q  E  L  C  G  G  A  L  Q  L  L

TGGGTTACCGGTCTGTGCATTCGGAGCTTGAGCAAAGGGACCTCTCTCTGGACCTCCTGC  1200
  G  Y  R  S  V  H  S  E  L  E  Q  R  D  L  S  L  D  L  L  L

TGCCAAGAGGCATGGACAGTTTCAAGTGGGCATCGTCCACGGAGAAGCAACCGGAATCTT  1260
  P  R  G  M  D  S  F  K  W  A  S  S  T  E  K  Q  P  E  S  *

AGAATTTTAGTGGAGAGACCCAGCTATAACATTAGGGTCTATTGGAGTATGATAAAGAAG  1320

GGGCTTGGAGAACCCAAAAGCAAGTAGCTGGGAGTGTGAGTGATCTTGTCCTGTACTAGG  1380

AAAGGATGGAGTCCAAATCCCACATCTCTTTCTGTCCAGATTGTAGTTTTCGGTTTTGGT  1440

CTTTTAGGGTTTGGATTCCCACCAAGTACTATCGAATGGAAAGCAAAAGCTGTGCCCACT  1500

TCCTTCAGAGAGGCAGCCAGCCTCCTACTAAAGCACTTCCTTTCTCGTTGACTCTCTCCC  1560

CTCTTTGATCATACCATGCAATCGCAGAGAATGGGGTCCCAGGCCTGCTCTGGAGTGCGG  1620

GAAAGGCGCGGCTGTGGGCTGGCTCCTAAAATCTGTGCACCTGCCTCTCGTTGGCTCACC  1680

CAGACCTCTGCTCACTGCCACGCCCTAGTATCTCAGTCCATCATAGACTTGGACAGTTAT  1740

GGGCCTGGTCAAGGAGGAAAATGAGACGATGCTTCCCTCTGTGATTCTCTGCCTGACCTT  1800

CTAGAAGGGAATCCAGGCACACACACAACCATACCTGAGGAGGATGGCTTTTTAATGAAT  1860

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FIG. 10 CONT.

SEQUENCE LISTING

<110> The Burnham Institute
Fukuda, Minoru
Yeh, Jiunn-Chern
Hiraoka, Nobuyoshi

<120> Identification of the Meca-79 Antigen
and Related Methods of Treating L-Selectin-Mediated
Conditions

<130> FP-LJ 4631

<140> PCT/US01/15452
<141> 2001-05-10

<150> US 09/569,320
<151> 2000-05-11

<160> 29

<170> FastSEQ for Windows Version 4.0

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ctc att ctg gcc atc ggc gct ttc acc ctc ctc ctc ttc agt ctg cta 97
Leu Ile Leu Ala Ile Gly Ala Phe Thr Leu Leu Leu Phe Ser Leu Leu
15 20 25

gtg tca cca ccc acc tgc aag gtc cag gag cag cca ccg gcg atc ccc 145
Val Ser Pro Pro Thr Cys Lys Val Gln Glu Gln Pro Pro Ala Ile Pro
30 35 40

gag gcc ctg gcc tgg ccc act cca ccc acc cgc cca gcc ccg gcc ccg 193
Glu Ala Leu Ala Trp Pro Thr Pro Pro Thr Arg Pro Ala Pro Ala Pro
45 50 55 60

tgc cat gcc aac acc tct atg gtc acc cac ccg gac ttc gcc acg cag 241
Cys His Ala Asn Thr Ser Met Val Thr His Pro Asp Phe Ala Thr Gln
65 70 75

ccg cag cac gtt cag aac ttc ctc ctg tac aga cac tgc cgc cac ttt 289

```
Pro Gln His Val Gln Asn Phe Leu Leu Tyr Arg His Cys Arg His Phe
             80                  85                  90

ccc ctg ctg cag gac gtg ccc ccc tct aag tgc gcg cag ccg gtc ttc   337
Pro Leu Leu Gln Asp Val Pro Pro Ser Lys Cys Ala Gln Pro Val Phe
         95                 100                 105

ctg ctg ctg gtg atc aag tcc tcc cct agc aac tat gtg cgc cgc gag   385
Leu Leu Leu Val Ile Lys Ser Ser Pro Ser Asn Tyr Val Arg Arg Glu
    110                 115                 120

ctg ctg cgg cgc acg tgg ggc cgc gag cgc aag gta cgg ggt ttg cag   433
Leu Leu Arg Arg Thr Trp Gly Arg Glu Arg Lys Val Arg Gly Leu Gln
125                 130                 135                 140

ctg cgc ctc ctc ttc ctg gtg ggc aca gcc tcc aac ccg cac gag gcc   481
Leu Arg Leu Leu Phe Leu Val Gly Thr Ala Ser Asn Pro His Glu Ala
                145                 150                 155

cgc aag gtc aac cgg ctg ctg gag ctg gag gca cag act cac gga gac   529
Arg Lys Val Asn Arg Leu Leu Glu Leu Glu Ala Gln Thr His Gly Asp
            160                 165                 170

atc ctg cag tgg gac ttc cac gac tcc ttc ttc aac ctc acg ctc aag   577
Ile Leu Gln Trp Asp Phe His Asp Ser Phe Phe Asn Leu Thr Leu Lys
        175                 180                 185

cag gtc ctg ttc tta cag tgg cag gag aca agg tgc gcc aac gcc agc   625
Gln Val Leu Phe Leu Gln Trp Gln Glu Thr Arg Cys Ala Asn Ala Ser
    190                 195                 200

ttc gtg ctc aac ggg gat gat gac gtc ttt gca cac aca gac aac atg   673
Phe Val Leu Asn Gly Asp Asp Asp Val Phe Ala His Thr Asp Asn Met
205                 210                 215                 220

gtc ttc tac ctg cag gac cat gac cct ggc cgc cac ctc ttc gtg ggg   721
Val Phe Tyr Leu Gln Asp His Asp Pro Gly Arg His Leu Phe Val Gly
                225                 230                 235

caa ctg atc caa aac gtg ggc ccc atc cgg gct ttt tgg agc aag tac   769
Gln Leu Ile Gln Asn Val Gly Pro Ile Arg Ala Phe Trp Ser Lys Tyr
            240                 245                 250

tat gtg cca gag gtg gtg act cag aat gag cgg tac cca ccc tat tgt   817
Tyr Val Pro Glu Val Val Thr Gln Asn Glu Arg Tyr Pro Pro Tyr Cys
        255                 260                 265

ggg ggt ggt ggc ttc ttg ctg tcc cgc ttc acg gcc gct gcc ctg cgc   865
Gly Gly Gly Gly Phe Leu Leu Ser Arg Phe Thr Ala Ala Ala Leu Arg
    270                 275                 280

cgt gct gcc cat gtc ttg gac atc ttc ccc att gat gat gtc ttc ctg   913
Arg Ala Ala His Val Leu Asp Ile Phe Pro Ile Asp Asp Val Phe Leu
285                 290                 295                 300
```

```
ggt atg tgt ctg gag ctt gag gga ctg aag cct gcc tcc cac agc ggc   961
Gly Met Cys Leu Glu Leu Glu Gly Leu Lys Pro Ala Ser His Ser Gly
                305                 310                 315

atc cgc acg tct ggc gtg cgg gct cca tcg caa cac ctg tcc tcc ttt   1009
Ile Arg Thr Ser Gly Val Arg Ala Pro Ser Gln His Leu Ser Ser Phe
            320                 325                 330

gac ccc tgc ttc tac cga gac ctg ctg ctg gtg cac cgc ttc cta cct   1057
Asp Pro Cys Phe Tyr Arg Asp Leu Leu Leu Val His Arg Phe Leu Pro
        335                 340                 345

tat gag atg ctg ctc atg tgg gat gcg ctg aac cag ccc aac ctc acc   1105
Tyr Glu Met Leu Leu Met Trp Asp Ala Leu Asn Gln Pro Asn Leu Thr
    350                 355                 360

tgc ggc aat cag aca cag atc tac tgagtcagca tcaggcatcc gcacgtctgg  1159
Cys Gly Asn Gln Thr Gln Ile Tyr
365                 370

cgtgcgggct ccatcgcaac acctgtcctc ctttgacccc tgcttctac             1208

<210> 2
<211> 372
<212> PRT
<213> Homo Sapien

<400> 2
Met Lys Tyr Leu Arg His Arg Arg Pro Asn Ala Thr Leu Ile Leu Ala
 1               5                  10                  15
Ile Gly Ala Phe Thr Leu Leu Leu Phe Ser Leu Leu Val Ser Pro Pro
            20                  25                  30
Thr Cys Lys Val Gln Glu Gln Pro Pro Ala Ile Pro Glu Ala Leu Ala
        35                  40                  45
Trp Pro Thr Pro Pro Thr Arg Pro Ala Pro Ala Pro Cys His Ala Asn
    50                  55                  60
Thr Ser Met Val Thr His Pro Asp Phe Ala Thr Gln Pro Gln His Val
65                  70                  75                  80
Gln Asn Phe Leu Leu Tyr Arg His Cys Arg His Phe Pro Leu Leu Gln
                85                  90                  95
Asp Val Pro Pro Ser Lys Cys Ala Gln Pro Val Phe Leu Leu Leu Val
            100                 105                 110
Ile Lys Ser Ser Pro Ser Asn Tyr Val Arg Arg Glu Leu Leu Arg Arg
        115                 120                 125
Thr Trp Gly Arg Glu Arg Lys Val Arg Gly Leu Gln Leu Arg Leu Leu
    130                 135                 140
Phe Leu Val Gly Thr Ala Ser Asn Pro His Glu Ala Arg Lys Val Asn
145                 150                 155                 160
Arg Leu Leu Glu Leu Glu Ala Gln Thr His Gly Asp Ile Leu Gln Trp
                165                 170                 175
Asp Phe His Asp Ser Phe Phe Asn Leu Thr Leu Lys Gln Val Leu Phe
            180                 185                 190
Leu Gln Trp Gln Glu Thr Arg Cys Ala Asn Ala Ser Phe Val Leu Asn
        195                 200                 205
Gly Asp Asp Asp Val Phe Ala His Thr Asp Asn Met Val Phe Tyr Leu
```

```
    210                 215                 220
Gln Asp His Asp Pro Gly Arg His Leu Phe Val Gly Gln Leu Ile Gln
225                 230                 235                 240
Asn Val Gly Pro Ile Arg Ala Phe Trp Ser Lys Tyr Tyr Val Pro Glu
                245                 250                 255
Val Val Thr Gln Asn Glu Arg Tyr Pro Pro Tyr Cys Gly Gly Gly Gly
            260                 265                 270
Phe Leu Leu Ser Arg Phe Thr Ala Ala Ala Leu Arg Arg Ala Ala His
        275                 280                 285
Val Leu Asp Ile Phe Pro Ile Asp Asp Val Phe Leu Gly Met Cys Leu
    290                 295                 300
Glu Leu Glu Gly Leu Lys Pro Ala Ser His Ser Gly Ile Arg Thr Ser
305                 310                 315                 320
Gly Val Arg Ala Pro Ser Gln His Leu Ser Ser Phe Asp Pro Cys Phe
                325                 330                 335
Tyr Arg Asp Leu Leu Leu Val His Arg Phe Leu Pro Tyr Glu Met Leu
            340                 345                 350
Leu Met Trp Asp Ala Leu Asn Gln Pro Asn Leu Thr Cys Gly Asn Gln
        355                 360                 365
Thr Gln Ile Tyr
    370
```

<210> 3
<211> 1337
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (19)...(1122)

<400> 3

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aggctccgcc cccacgcc atg cgg ctg cca agg cag agc ccc tac gag atc   51
                      Met Arg Leu Pro Arg Gln Ser Pro Tyr Glu Ile
                       1               5                  10

ctc ctc ctg gtc ttg gtc gcc ttg ctg gtg ctg ctg ctg ctc ctg acc   99
Leu Leu Leu Val Leu Val Ala Leu Leu Val Leu Leu Leu Leu Leu Thr
             15                  20                  25

agc aag tca ccg ccc agc tgc tcc gcc cct gag agg tcc aag gag cct   147
Ser Lys Ser Pro Pro Ser Cys Ser Ala Pro Glu Arg Ser Lys Glu Pro
         30                  35                  40

gaa gac aac ccc ggg tgg gcc acg ggc cac ccc gcc cgg tgc cga gct   195
Glu Asp Asn Pro Gly Trp Ala Thr Gly His Pro Ala Arg Cys Arg Ala
     45                  50                  55

aat cta tcc gtg tcc tcg cac ccc gac ttc gcg ggg ctg ccc ttg cac   243
Asn Leu Ser Val Ser Ser His Pro Asp Phe Ala Gly Leu Pro Leu His
 60                  65                  70                  75

gtg cgc gac ttc ttg ttc tac cgc cac tgc cgc gac ttc cca gtg ctc   291
Val Arg Asp Phe Leu Phe Tyr Arg His Cys Arg Asp Phe Pro Val Leu
```

```
                80                  85                  90

cga gag ccg cgg gtt acc aag tgc gcg gag ccc gtg ttc ctg ctg ctc   339
Arg Glu Pro Arg Val Thr Lys Cys Ala Glu Pro Val Phe Leu Leu Leu
            95                 100                 105

gcc atc aag tcc tcg cct gca aac tat ggg cgc cgc cag atg ctg cgc   387
Ala Ile Lys Ser Ser Pro Ala Asn Tyr Gly Arg Arg Gln Met Leu Arg
        110                 115                 120

acg acg tgg gcg cgc gag aga cgg gtg cgt ggg gcg cca ctg cgc cgc   435
Thr Thr Trp Ala Arg Glu Arg Arg Val Arg Gly Ala Pro Leu Arg Arg
    125                 130                 135

ctc ttc ctt gtg ggc tca gac cgc gac cca caa caa gca cgc aaa tac   483
Leu Phe Leu Val Gly Ser Asp Arg Asp Pro Gln Gln Ala Arg Lys Tyr
140                 145                 150                 155

aac cga ctg ctg gag ctg gaa gcg cag aaa tac ggc gac att ctc cag   531
Asn Arg Leu Leu Glu Leu Glu Ala Gln Lys Tyr Gly Asp Ile Leu Gln
                160                 165                 170

tgg gat ttc cat gac tcc ttc ttt aac ctg acg ctt aag cag gtc ctt   579
Trp Asp Phe His Asp Ser Phe Phe Asn Leu Thr Leu Lys Gln Val Leu
            175                 180                 185

ttc ctg gag tgg cag cta acc tac tgt acc aac gcc agc ttc gtg ctc   627
Phe Leu Glu Trp Gln Leu Thr Tyr Cys Thr Asn Ala Ser Phe Val Leu
        190                 195                 200

aat ggg gac gac gat gtg ttc gca cac acg gac aac atg gtc acc tac   675
Asn Gly Asp Asp Asp Val Phe Ala His Thr Asp Asn Met Val Thr Tyr
    205                 210                 215

ctg cag gac cac gac ccg gac caa cac ctc ttc gtg ggg cac ctg atc   723
Leu Gln Asp His Asp Pro Asp Gln His Leu Phe Val Gly His Leu Ile
220                 225                 230                 235

cag aac gtg ggt ccc atc cgg gtg ccc tgg agc aag tac ttc atc ccc   771
Gln Asn Val Gly Pro Ile Arg Val Pro Trp Ser Lys Tyr Phe Ile Pro
                240                 245                 250

gct ctg gtg atg gcg gag gac aga tac ccg ccc tac tgt ggt ggc ggc   819
Ala Leu Val Met Ala Glu Asp Arg Tyr Pro Pro Tyr Cys Gly Gly Gly
            255                 260                 265

ggc ttc ctg ctg tcg cgt ttt acc gtg gcc gcc cta cgt cgc gcc gcg   867
Gly Phe Leu Leu Ser Arg Phe Thr Val Ala Ala Leu Arg Arg Ala Ala
        270                 275                 280

cgc gtc ctc ccc atg ttc cca atc gac gac gtg ttc ctg ggc atg tgt   915
Arg Val Leu Pro Met Phe Pro Ile Asp Asp Val Phe Leu Gly Met Cys
    285                 290                 295

ctg cag cag cag ggt ctg gct ccc ggg acg cac agc gga gtg cgc act   963
```

```
Leu Gln Gln Gln Gly Leu Ala Pro Gly Thr His Ser Gly Val Arg Thr
300                 305                 310                 315

gcg ggg gtt ttc ccc cct agc cca cgt gtg tca tcc ttc gac ccc tgc   1011
Ala Gly Val Phe Pro Pro Ser Pro Arg Val Ser Ser Phe Asp Pro Cys
                320                 325                 330

ttc tac cgc gac ctg ctc ctc gtg cac cgc ttc ctg ccc ttc gag atg   1059
Phe Tyr Arg Asp Leu Leu Leu Val His Arg Phe Leu Pro Phe Glu Met
            335                 340                 345

ctg ctg atg tgg gat gcg ctg aac cag ccc cag ctc ctc tgc ggc agg   1107
Leu Leu Met Trp Asp Ala Leu Asn Gln Pro Gln Leu Leu Cys Gly Arg
        350                 355                 360

cag agc ccc gcc tac tgagaggttt gggggagttg acatccccta gctcatgtcc   1162
Gln Ser Pro Ala Tyr
    365

tgcctcatcc acgtgcaaag ggctggcttc aaggagaagt tcaaagtgag gggcagaaag 1222
gtgggtctga ggagttcata gggcaaactc ctaagtacgc ttggaaaccc tcttggtact 1282
gttcacagca ggaactctga gtctagccaa ctctgagtgg ctctaagtgg ccgct      1337

<210> 4
<211> 368
<212> PRT
<213> Mus musculus

<400> 4
Met Arg Leu Pro Arg Gln Ser Pro Tyr Glu Ile Leu Leu Leu Val Leu
 1               5                  10                  15
Val Ala Leu Leu Val Leu Leu Leu Leu Leu Thr Ser Lys Ser Pro Pro
            20                  25                  30
Ser Cys Ser Ala Pro Glu Arg Ser Lys Glu Pro Glu Asp Asn Pro Gly
        35                  40                  45
Trp Ala Thr Gly His Pro Ala Arg Cys Arg Ala Asn Leu Ser Val Ser
    50                  55                  60
Ser His Pro Asp Phe Ala Gly Leu Pro Leu His Val Arg Asp Phe Leu
65                  70                  75                  80
Phe Tyr Arg His Cys Arg Asp Phe Pro Val Leu Arg Glu Pro Arg Val
                85                  90                  95
Thr Lys Cys Ala Glu Pro Val Phe Leu Leu Leu Ala Ile Lys Ser Ser
            100                 105                 110
Pro Ala Asn Tyr Gly Arg Arg Gln Met Leu Arg Thr Thr Trp Ala Arg
        115                 120                 125
Glu Arg Arg Val Arg Gly Ala Pro Leu Arg Arg Leu Phe Leu Val Gly
    130                 135                 140
Ser Asp Arg Asp Pro Gln Gln Ala Arg Lys Tyr Asn Arg Leu Leu Glu
145                 150                 155                 160
Leu Glu Ala Gln Lys Tyr Gly Asp Ile Leu Gln Trp Asp Phe His Asp
                165                 170                 175
Ser Phe Phe Asn Leu Thr Leu Lys Gln Val Leu Phe Leu Glu Trp Gln
            180                 185                 190
Leu Thr Tyr Cys Thr Asn Ala Ser Phe Val Leu Asn Gly Asp Asp Asp
        195                 200                 205
```

```
Val Phe Ala His Thr Asp Asn Met Val Thr Tyr Leu Gln Asp His Asp
    210                 215                 220
Pro Asp Gln His Leu Phe Val Gly His Leu Ile Gln Asn Val Gly Pro
225                 230                 235                 240
Ile Arg Val Pro Trp Ser Lys Tyr Phe Ile Pro Ala Leu Val Met Ala
                245                 250                 255
Glu Asp Arg Tyr Pro Pro Tyr Cys Gly Gly Gly Gly Phe Leu Leu Ser
            260                 265                 270
Arg Phe Thr Val Ala Ala Leu Arg Arg Ala Ala Arg Val Leu Pro Met
        275                 280                 285
Phe Pro Ile Asp Asp Val Phe Leu Gly Met Cys Leu Gln Gln Gln Gly
    290                 295                 300
Leu Ala Pro Gly Thr His Ser Gly Val Arg Thr Ala Gly Val Phe Pro
305                 310                 315                 320
Pro Ser Pro Arg Val Ser Ser Phe Asp Pro Cys Phe Tyr Arg Asp Leu
                325                 330                 335
Leu Leu Val His Arg Phe Leu Pro Phe Glu Met Leu Leu Met Trp Asp
            340                 345                 350
Ala Leu Asn Gln Pro Gln Leu Leu Cys Gly Arg Gln Ser Pro Ala Tyr
        355                 360                 365
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<210> 5
<211> 1333
<212> DNA
<213> Homo Sapien

<220>
<221> CDS
<222> (111)...(1250)

<400> 5

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ttggccagaa ggggaataga aggcaaacaa taaaacagca gcccaactcc acccttttctg 60
tttgttcctt aaaggtcttc cacttcagca caatgctact gcctaaaaaa atg aag    116
                                                          Met Lys
                                                           1

ctc ctg ctg ttt ctg gtt tcc cag atg gcc atc ttg gct cta ttc ttc   164
Leu Leu Leu Phe Leu Val Ser Gln Met Ala Ile Leu Ala Leu Phe Phe
 5                  10                  15

cac atg tac agc cac aac atc agc tcc ctg tct atg aag gca cag ccc   212
His Met Tyr Ser His Asn Ile Ser Ser Leu Ser Met Lys Ala Gln Pro
 20                  25                  30

gag cgc atg cac gtg ctg gtt ctg tct tcc tgg cgc tct ggc tct tct   260
Glu Arg Met His Val Leu Val Leu Ser Ser Trp Arg Ser Gly Ser Ser
 35                  40                  45                  50

ttt gtg ggg cag ctt ttt ggg cag cac cca gat gtt ttc tac ctg atg   308
Phe Val Gly Gln Leu Phe Gly Gln His Pro Asp Val Phe Tyr Leu Met
                55                  60                  65

gag ccc gcc tgg cac gtg tgg atg acc ttc aag cag agc acc gcc tgg   356
Glu Pro Ala Trp His Val Trp Met Thr Phe Lys Gln Ser Thr Ala Trp
```

```
                70                  75                  80

atg ctg cac atg gct gtg cgg gat ctg ata cgg gcc gtc ttc ttg tgc   404
Met Leu His Met Ala Val Arg Asp Leu Ile Arg Ala Val Phe Leu Cys
         85                  90                  95

gac atg agc gtc ttt gat gcc tac atg gaa cct ggt ccc cgg aga cag   452
Asp Met Ser Val Phe Asp Ala Tyr Met Glu Pro Gly Pro Arg Arg Gln
    100                 105                 110

tcc agc ctc ttt cag tgg gag aac agc cgg gcc ctg tgt tct gca cct   500
Ser Ser Leu Phe Gln Trp Glu Asn Ser Arg Ala Leu Cys Ser Ala Pro
115                 120                 125                 130

gcc tgt gac atc atc cca caa gat gaa atc atc ccc cgg gct cac tgc   548
Ala Cys Asp Ile Ile Pro Gln Asp Glu Ile Ile Pro Arg Ala His Cys
                135                 140                 145

agg ctc ctg tgc agt caa cag ccc ttt gag gtg gtg gag aag gcc tgc   596
Arg Leu Leu Cys Ser Gln Gln Pro Phe Glu Val Val Glu Lys Ala Cys
            150                 155                 160

cgc tcc tac agc cac gtg gtg ctc aag gag gtg cgc ttc ttc aac ctg   644
Arg Ser Tyr Ser His Val Val Leu Lys Glu Val Arg Phe Phe Asn Leu
        165                 170                 175

cag tcc ctc tac ccg ctg ctg aaa gac ccc tcc ctc aac ctg cat atc   692
Gln Ser Leu Tyr Pro Leu Leu Lys Asp Pro Ser Leu Asn Leu His Ile
    180                 185                 190

gtg cac ctg gtc cgg gac ccc cgg gcc gtg ttc cgt tcc cga gaa cgc   740
Val His Leu Val Arg Asp Pro Arg Ala Val Phe Arg Ser Arg Glu Arg
195                 200                 205                 210

aca aag gga gat ctc atg att gac agt cgc att gtg atg ggg cag cat   788
Thr Lys Gly Asp Leu Met Ile Asp Ser Arg Ile Val Met Gly Gln His
                215                 220                 225

gag caa aaa ctc aag aag gag gac caa ccc tac tat gtg atg cag gtc   836
Glu Gln Lys Leu Lys Lys Glu Asp Gln Pro Tyr Tyr Val Met Gln Val
            230                 235                 240

atc tgc caa agc cag ctg gag atc tac aag acc atc cag tcc ttg ccc   884
Ile Cys Gln Ser Gln Leu Glu Ile Tyr Lys Thr Ile Gln Ser Leu Pro
        245                 250                 255

aag gcc ctg cag gaa cgc tac ctg ctt gtg cgc tat gag gac ctg gct   932
Lys Ala Leu Gln Glu Arg Tyr Leu Leu Val Arg Tyr Glu Asp Leu Ala
    260                 265                 270

cga gcc cct gtg gcc cag act tcc cga atg tat gaa ttc gtg gga ttg   980
Arg Ala Pro Val Ala Gln Thr Ser Arg Met Tyr Glu Phe Val Gly Leu
275                 280                 285                 290

gaa ttc ttg ccc cat ctt cag acc tgg gtg cat aac atc acc cga ggc   1028
```

```
Glu Phe Leu Pro His Leu Gln Thr Trp Val His Asn Ile Thr Arg Gly
                295                 300                 305

aag ggc atg ggt gac cac gct ttc cac aca aat gcc agg gat gcc ctt   1076
Lys Gly Met Gly Asp His Ala Phe His Thr Asn Ala Arg Asp Ala Leu
            310                 315                 320

aat gtc tcc cag gct tgg cgc tgg tct ttg ccc tat gaa aag gtt tct   1124
Asn Val Ser Gln Ala Trp Arg Trp Ser Leu Pro Tyr Glu Lys Val Ser
        325                 330                 335

cga ctt cag aaa gcc tgt ggc gat gcc atg aat ttg ctg ggc tac cgc   1172
Arg Leu Gln Lys Ala Cys Gly Asp Ala Met Asn Leu Leu Gly Tyr Arg
    340                 345                 350

cac gtc aga tct gaa caa gaa cag aga aac ctg ttg ctg gat ctt ctg   1220
His Val Arg Ser Glu Gln Glu Gln Arg Asn Leu Leu Leu Asp Leu Leu
355                 360                 365                 370

tct acc tgg act gtc cct gag caa atc cac taagagggtt gagaaggctt     1270
Ser Thr Trp Thr Val Pro Glu Gln Ile His
                375                 380

tgctgccacc tggtgtcagc ctcagtcact ttctctgaat gcttctgagc cttgcctaca 1330
tct                                                               1333

<210> 6
<211> 380
<212> PRT
<213> Homo Sapien

<400> 6
Met Lys Leu Leu Leu Phe Leu Val Ser Gln Met Ala Ile Leu Ala Leu
 1               5                  10                  15
Phe Phe His Met Tyr Ser His Asn Ile Ser Ser Leu Ser Met Lys Ala
            20                  25                  30
Gln Pro Glu Arg Met His Val Leu Val Leu Ser Ser Trp Arg Ser Gly
        35                  40                  45
Ser Ser Phe Val Gly Gln Leu Phe Gly Gln His Pro Asp Val Phe Tyr
    50                  55                  60
Leu Met Glu Pro Ala Trp His Val Trp Met Thr Phe Lys Gln Ser Thr
65                  70                  75                  80
Ala Trp Met Leu His Met Ala Val Arg Asp Leu Ile Arg Ala Val Phe
                85                  90                  95
Leu Cys Asp Met Ser Val Phe Asp Ala Tyr Met Glu Pro Gly Pro Arg
            100                 105                 110
Arg Gln Ser Ser Leu Phe Gln Trp Glu Asn Ser Arg Ala Leu Cys Ser
        115                 120                 125
Ala Pro Ala Cys Asp Ile Ile Pro Gln Asp Glu Ile Ile Pro Arg Ala
    130                 135                 140
His Cys Arg Leu Leu Cys Ser Gln Gln Pro Phe Glu Val Val Glu Lys
145                 150                 155                 160
Ala Cys Arg Ser Tyr Ser His Val Val Leu Lys Glu Val Arg Phe Phe
                165                 170                 175
Asn Leu Gln Ser Leu Tyr Pro Leu Leu Lys Asp Pro Ser Leu Asn Leu
```

```
            180                 185                 190
His Ile Val His Leu Val Arg Asp Pro Arg Ala Val Phe Arg Ser Arg
        195                 200                 205
Glu Arg Thr Lys Gly Asp Leu Met Ile Asp Ser Arg Ile Val Met Gly
    210                 215                 220
Gln His Glu Gln Lys Leu Lys Lys Glu Asp Gln Pro Tyr Tyr Val Met
225                 230                 235                 240
Gln Val Ile Cys Gln Ser Gln Leu Glu Ile Tyr Lys Thr Ile Gln Ser
                245                 250                 255
Leu Pro Lys Ala Leu Gln Glu Arg Tyr Leu Leu Val Arg Tyr Glu Asp
            260                 265                 270
Leu Ala Arg Ala Pro Val Ala Gln Thr Ser Arg Met Tyr Glu Phe Val
        275                 280                 285
Gly Leu Glu Phe Leu Pro His Leu Gln Thr Trp Val His Asn Ile Thr
    290                 295                 300
Arg Gly Lys Gly Met Gly Asp His Ala Phe His Thr Asn Ala Arg Asp
305                 310                 315                 320
Ala Leu Asn Val Ser Gln Ala Trp Arg Trp Ser Leu Pro Tyr Glu Lys
                325                 330                 335
Val Ser Arg Leu Gln Lys Ala Cys Gly Asp Ala Met Asn Leu Leu Gly
            340                 345                 350
Tyr Arg His Val Arg Ser Glu Gln Glu Gln Arg Asn Leu Leu Leu Asp
        355                 360                 365
Leu Leu Ser Thr Trp Thr Val Pro Glu Gln Ile His
    370                 375                 380
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<210> 7
<211> 1937
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (75)...(1259)

<400> 7

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tgagcggctc tttgtgtgcg ccctgggtgc gcagcgcaga agcgcagcgg gcagcgcagg 60
ccctagccag aggt atg cgg cta ccc cgt ttc tcc agc act gtc atg ctt  110
                Met Arg Leu Pro Arg Phe Ser Ser Thr Val Met Leu
                 1               5                  10

tcg ctc ctg atg gta cag act ggc atc ctg gtc ttc ctg gtc tcc cgg  158
Ser Leu Leu Met Val Gln Thr Gly Ile Leu Val Phe Leu Val Ser Arg
         15                  20                  25

caa gtg cca tcg tcc cca gca ggc ctt ggg gag cgt gtg cac gtg ctg  206
Gln Val Pro Ser Ser Pro Ala Gly Leu Gly Glu Arg Val His Val Leu
     30                  35                  40

gta ctg tcc tcg tgg cgc tcg ggc tcg tcc ttc gtg ggc cag ctc ttc  254
Val Leu Ser Ser Trp Arg Ser Gly Ser Ser Phe Val Gly Gln Leu Phe
 45                  50                  55                  60

agc caa cac ccc gat gtc ttc tac ctg atg gag ccg gct tgg cac gtc  302
```

```
Ser Gln His Pro Asp Val Phe Tyr Leu Met Glu Pro Ala Trp His Val
                65                  70                  75

tgg gat acg ttg tcg cag ggc agt gcc ccc gca ctc cac atg gcc gtg   350
Trp Asp Thr Leu Ser Gln Gly Ser Ala Pro Ala Leu His Met Ala Val
            80                  85                  90

cgt gac ctg atc cgc tca gtg ttc cta tgc gac atg gac gta ttt gat   398
Arg Asp Leu Ile Arg Ser Val Phe Leu Cys Asp Met Asp Val Phe Asp
        95                  100                 105

gcc tac ctg ccc tgg cgc cgc aac atc tcg gat ctc ttc cag tgg gcg   446
Ala Tyr Leu Pro Trp Arg Arg Asn Ile Ser Asp Leu Phe Gln Trp Ala
    110                 115                 120

gtg agc cgc gca ttg tgc tca cct ccg gtc tgc gaa gcc ttc gct cgt   494
Val Ser Arg Ala Leu Cys Ser Pro Pro Val Cys Glu Ala Phe Ala Arg
125                 130                 135                 140

ggc aac atc agc agc gag gag gtg tgt aag cct ctg tgc gca acg cgg   542
Gly Asn Ile Ser Ser Glu Glu Val Cys Lys Pro Leu Cys Ala Thr Arg
                145                 150                 155

ccc ttc ggc ctg gct cag gaa gcc tgc agc tcc tat agt cac gtc gtg   590
Pro Phe Gly Leu Ala Gln Glu Ala Cys Ser Ser Tyr Ser His Val Val
            160                 165                 170

ctc aag gag gtg cgc ttc ttt aac cta cag gtg ctc tac ccg ctg ctc   638
Leu Lys Glu Val Arg Phe Phe Asn Leu Gln Val Leu Tyr Pro Leu Leu
        175                 180                 185

agc gac cct gcg ctc aac ctg cgc atc gtg cac cta gtg cgc gac ccg   686
Ser Asp Pro Ala Leu Asn Leu Arg Ile Val His Leu Val Arg Asp Pro
    190                 195                 200

cgg gcc gtg ctg cgc tcc cga gag cag aca gcc aag gcg ctg gca cgg   734
Arg Ala Val Leu Arg Ser Arg Glu Gln Thr Ala Lys Ala Leu Ala Arg
205                 210                 215                 220

gac aat ggc atc gtc ctg ggt acc aac ggc acg tgg gtg gag gcg gac   782
Asp Asn Gly Ile Val Leu Gly Thr Asn Gly Thr Trp Val Glu Ala Asp
                225                 230                 235

ccc cgg ctg cgc gtg gtc aac gag gta tgc cgc agc cat gtg cgc atc   830
Pro Arg Leu Arg Val Val Asn Glu Val Cys Arg Ser His Val Arg Ile
            240                 245                 250

gca gag gca gcc ttg cac aag ccg ccg ccc ttc ttg caa gat cgc tac   878
Ala Glu Ala Ala Leu His Lys Pro Pro Pro Phe Leu Gln Asp Arg Tyr
        255                 260                 265

cgc ctg gtg cgc tac gag gat ctg gcc cgg gac cca ctc acc gta atc   926
Arg Leu Val Arg Tyr Glu Asp Leu Ala Arg Asp Pro Leu Thr Val Ile
    270                 275                 280
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cgt gaa ctc tat gcc ttc acc ggc ctg ggt ctc acg ccg cag ctc cag   974
Arg Glu Leu Tyr Ala Phe Thr Gly Leu Gly Leu Thr Pro Gln Leu Gln
285                 290                 295                 300

act tgg atc cac aat atc acg cat ggt tca ggg cca ggc gcg cgc cgt   1022
Thr Trp Ile His Asn Ile Thr His Gly Ser Gly Pro Gly Ala Arg Arg
                305                 310                 315

gaa gcc ttc aag acc aca tcc agg gat gcg ctc agt gta tcc cag gcc   1070
Glu Ala Phe Lys Thr Thr Ser Arg Asp Ala Leu Ser Val Ser Gln Ala
            320                 325                 330

tgg cgc cac acg ctg ccc ttt gcc aag att cgc cgg gtc cag gaa ctg   1118
Trp Arg His Thr Leu Pro Phe Ala Lys Ile Arg Arg Val Gln Glu Leu
        335                 340                 345

tgc ggg ggt gca ctg cag ctg ctg ggt tac cgg tct gtg cat tcg gag   1166
Cys Gly Gly Ala Leu Gln Leu Leu Gly Tyr Arg Ser Val His Ser Glu
    350                 355                 360

ctt gag caa agg gac ctc tct ctg gac ctc ctg ctg cca aga ggc atg   1214
Leu Glu Gln Arg Asp Leu Ser Leu Asp Leu Leu Leu Pro Arg Gly Met
365                 370                 375                 380

gac agt ttc aag tgg gca tcg tcc acg gag aag caa ccg gaa tct       1259
Asp Ser Phe Lys Trp Ala Ser Ser Thr Glu Lys Gln Pro Glu Ser
                385                 390                 395

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Ser Pro Ala Gly Leu Gly Glu Arg Val His Val Leu Val Leu Ser Ser
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Trp Arg Ser Gly Ser Ser Phe Val Gly Gln Leu Phe Ser Gln His Pro
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Ser Gln Gly Ser Ala Pro Ala Leu His Met Ala Val Arg Asp Leu Ile
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Arg Ser Val Phe Leu Cys Asp Met Asp Val Phe Asp Ala Tyr Leu Pro
            100                 105                 110
Trp Arg Arg Asn Ile Ser Asp Leu Phe Gln Trp Ala Val Ser Arg Ala
        115                 120                 125
Leu Cys Ser Pro Pro Val Cys Glu Ala Phe Ala Arg Gly Asn Ile Ser
    130                 135                 140
Ser Glu Glu Val Cys Lys Pro Leu Cys Ala Thr Arg Pro Phe Gly Leu
145                 150                 155                 160
Ala Gln Glu Ala Cys Ser Ser Tyr Ser His Val Val Leu Lys Glu Val
                165                 170                 175
Arg Phe Phe Asn Leu Gln Val Leu Tyr Pro Leu Leu Ser Asp Pro Ala
            180                 185                 190
Leu Asn Leu Arg Ile Val His Leu Val Arg Asp Pro Arg Ala Val Leu
        195                 200                 205
Arg Ser Arg Glu Gln Thr Ala Lys Ala Leu Ala Arg Asp Asn Gly Ile
    210                 215                 220
Val Leu Gly Thr Asn Gly Thr Trp Val Glu Ala Asp Pro Arg Leu Arg
225                 230                 235                 240
Val Val Asn Glu Val Cys Arg Ser His Val Arg Ile Ala Glu Ala Ala
                245                 250                 255
Leu His Lys Pro Pro Pro Phe Leu Gln Asp Arg Tyr Arg Leu Val Arg
            260                 265                 270
Tyr Glu Asp Leu Ala Arg Asp Pro Leu Thr Val Ile Arg Glu Leu Tyr
        275                 280                 285
Ala Phe Thr Gly Leu Gly Leu Thr Pro Gln Leu Gln Thr Trp Ile His
    290                 295                 300
Asn Ile Thr His Gly Ser Gly Pro Gly Ala Arg Arg Glu Ala Phe Lys
305                 310                 315                 320
Thr Thr Ser Arg Asp Ala Leu Ser Val Ser Gln Ala Trp Arg His Thr
                325                 330                 335
Leu Pro Phe Ala Lys Ile Arg Arg Val Gln Glu Leu Cys Gly Gly Ala
            340                 345                 350
Leu Gln Leu Leu Gly Tyr Arg Ser Val His Ser Glu Leu Glu Gln Arg
        355                 360                 365
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Thr Gly Ser Lys Pro Phe Ser His Leu Thr Val Ala Arg Lys Asn Phe
        35                  40                  45
Thr Phe Gly Asn Ile Arg Thr Arg Pro Ile Asn Pro His Ser Phe Glu
    50                  55                  60
Phe Leu Ile Asn Glu Pro Asn Lys Cys Glu Lys Asn Ile Pro Phe Leu
65                  70                  75                  80
Val Ile Leu Ile Ser Thr Thr His Lys Glu Phe Asp Ala Arg Gln Ala
                85                  90                  95
Ile Arg Glu Thr Trp Gly Asp Glu Asn Asn Phe Lys Gly Ile Lys Ile
            100                 105                 110
Ala Thr Leu Phe Leu Leu Gly Lys Asn Ala Asp Pro Val Leu Asn Gln
        115                 120                 125
Met Val Glu Gln Glu Ser Gln Ile Phe His Asp Ile Ile Val Glu Asp
    130                 135                 140
Phe Ile Asp Ser Tyr His Asn Leu Thr Leu Lys Thr Leu Met Gly Met
145                 150                 155                 160
Arg Trp Val Ala Thr Phe Cys Ser Lys Ala Lys Tyr Val Met Lys Thr
                165                 170                 175
Asp Ser Asp Ile Phe Val Asn Met Asp Asn Leu Ile Tyr Lys Leu Leu
            180                 185                 190
Lys Pro Ser Thr Lys Pro Arg Arg Arg Tyr Phe Thr Gly Tyr Val Ile
        195                 200                 205
Asn Gly Gly Pro Ile Arg Asp Val Arg Ser Lys Trp Tyr Met Pro Arg
    210                 215                 220
Asp Leu Tyr Pro Asp Ser Asn Tyr Pro Pro Phe Cys Ser Gly Thr Gly
225                 230                 235                 240
Tyr Ile Phe Ser Ala Asp Val Ala Glu Leu Ile Tyr Lys Thr Ser Leu
                245                 250                 255
His Thr Arg Leu Leu His Leu Glu Asp Val Tyr Val Gly Leu Cys Leu
            260                 265                 270
Arg Lys Leu Gly Ile His Pro Phe Gln Asn Ser Gly Phe Asn His Trp
        275                 280                 285
```

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Lys Met Ala Tyr Ser Leu Cys Arg Tyr Arg Arg Val Ile Thr Val His
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Gln Ile Ser Pro Glu Glu Met His Arg Ile Trp Asn Asp Met Ser Ser
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Lys Lys His Leu Arg Cys
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<400> 26

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Leu Val Phe Leu Phe Ala Met Phe Leu Phe Phe Asn His His Asp Trp
        35                  40                  45
Leu Pro Gly Arg Pro Gly Phe Lys Glu Asn Pro Val Thr Tyr Thr Phe
    50                  55                  60
Arg Gly Phe Arg Ser Thr Lys Ser Glu Thr Asn His Ser Ser Leu Arg
65                  70                  75                  80
Thr Ile Trp Lys Glu Val Ala Pro Gln Thr Leu Arg Pro His Ile Ala
                85                  90                  95
Ser Asn Ser Ser Asn Thr Glu Leu Ser Pro Gln Gly Val Thr Gly Leu
            100                 105                 110
Gln Asn Thr Leu Ser Ala Asn Gly Ser Ile Tyr Asn Glu Lys Gly Thr
        115                 120                 125
Gly His Pro Asn Ser Tyr His Phe Lys Tyr Ile Ile Asn Glu Pro Glu
    130                 135                 140
Lys Cys Gln Glu Lys Ser Pro Phe Leu Ile Leu Leu Ile Ala Ala Glu
145                 150                 155                 160
Pro Gly Gln Ile Glu Ala Arg Arg Ala Ile Arg Gln Thr Trp Gly Asn
                165                 170                 175
Glu Thr Leu Ala Pro Gly Ile Gln Ile Ile Arg Val Phe Leu Leu Gly
            180                 185                 190
Ile Ser Ile Lys Leu Asn Gly Tyr Leu Gln His Ala Ile Gln Glu Glu
        195                 200                 205
Ser Arg Gln Tyr His Asp Ile Ile Gln Gln Glu Tyr Leu Asp Thr Tyr
    210                 215                 220
Tyr Asn Leu Thr Ile Lys Thr Leu Met Gly Met Asn Trp Val Ala Thr
225                 230                 235                 240
Tyr Cys Pro His Thr Pro Tyr Val Met Lys Thr Asp Ser Asp Met Phe
                245                 250                 255
Val Asn Thr Glu Tyr Leu Ile His Lys Leu Leu Lys Pro Asp Leu Pro
            260                 265                 270
Pro Arg His Asn Tyr Phe Thr Gly Tyr Leu Met Arg Gly Tyr Ala Pro
        275                 280                 285
Asn Arg Asn Lys Asp Ser Lys Trp Tyr Met Pro Pro Asp Leu Tyr Pro
    290                 295                 300
Ser Glu Arg Tyr Pro Val Phe Cys Ser Gly Thr Gly Tyr Val Phe Ser
305                 310                 315                 320
Gly Asp Leu Ala Glu Lys Ile Phe Lys Val Ser Leu Gly Ile Arg Arg
```

```
                325                 330                 335
Leu His Leu Glu Asp Val Tyr Val Gly Ile Cys Leu Ala Lys Leu Arg
            340                 345                 350
Val Asp Pro Val Pro Pro Pro Asn Glu Phe Val Phe Asn His Trp Arg
        355                 360                 365
Val Ser Tyr Ser Ser Cys Lys Tyr Ser His Leu Ile Thr Ser His Gln
    370                 375                 380
Phe Gln Pro Ser Glu Leu Ile Lys Tyr Trp Asn His Leu Gln Gln Asn
385                 390                 395                 400
Lys His Asn Ala Cys Ala Asn Ala Ala Lys Glu Lys Ala Gly Arg Tyr
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<400> 27

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Trp Met Tyr Phe Tyr Glu Tyr Glu Pro Ile Tyr Arg Gln Asp Phe Arg
    50                  55                  60
Phe Thr Leu Arg Glu His Ser Asn Cys Ser His Gln Asn Pro Phe Leu
65                  70                  75                  80
Val Ile Leu Val Thr Ser Arg Pro Ser Asp Val Lys Ala Arg Gln Ala
                85                  90                  95
Ile Arg Val Thr Trp Gly Glu Lys Lys Ser Trp Trp Gly Tyr Glu Val
            100                 105                 110
Leu Thr Phe Phe Leu Leu Gly Gln Gln Ala Glu Arg Glu Asp Lys Thr
        115                 120                 125
Leu Ala Leu Ser Leu Glu Asp Glu His Val Leu Tyr Gly Asp Ile Ile
    130                 135                 140
Arg Gln Asp Phe Leu Asp Thr Tyr Asn Asn Leu Thr Leu Lys Thr Ile
145                 150                 155                 160
Met Ala Phe Arg Trp Val Met Glu Phe Cys Pro Asn Ala Lys Tyr Ile
                165                 170                 175
Met Lys Thr Asp Thr Asp Val Phe Ile Asn Thr Gly Asn Leu Val Lys
            180                 185                 190
Tyr Leu Leu Asn Leu Asn His Ser Glu Lys Phe Phe Thr Gly Tyr Pro
        195                 200                 205
Leu Ile Asp Asn Tyr Ser Tyr Arg Gly Phe Phe His Lys Asn His Ile
    210                 215                 220
Ser Tyr Gln Glu Tyr Pro Phe Lys Val Phe Pro Pro Tyr Cys Ser Gly
225                 230                 235                 240
Leu Gly Tyr Ile Met Ser Gly Asp Leu Val Pro Arg Val Tyr Glu Met
                245                 250                 255
Met Ser His Val Lys Pro Ile Lys Phe Glu Asp Val Tyr Val Gly Ile
            260                 265                 270
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```
Cys Leu Asn Leu Leu Lys Val Asp Ile His Ile Pro Glu Asp Thr Asn
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Leu Phe Phe Leu Tyr Arg Ile His Leu Asp Val Cys Gln Leu Arg Arg
    290                 295                 300
Val Ile Ala Ala His Gly Phe Ser Ser Lys Glu Ile Ile Thr Phe Trp
305                 310                 315                 320
Gln Val Met Leu Arg Asn Thr Thr Cys His Tyr
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<400> 28

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            20                  25                  30
Leu Ser Leu Ser Leu Ala Ser Leu Leu Pro Ala Pro Ala Ser Pro Gly
        35                  40                  45
Pro Pro Leu Ala Leu Pro Arg Leu Leu Ile Ser Asn Ser His Ala Cys
    50                  55                  60
Gly Gly Ser Gly Pro Pro Pro Phe Leu Leu Ile Leu Val Cys Thr Ala
65                  70                  75                  80
Pro Glu His Leu Asn Gln Arg Asn Ala Ile Arg Ala Ser Trp Gly Ala
                85                  90                  95
Ile Arg Glu Ala Arg Gly Phe Arg Val Gln Thr Leu Phe Leu Leu Gly
            100                 105                 110
Lys Pro Arg Arg Gln Gln Leu Ala Asp Leu Ser Ser Glu Ser Ala Ala
        115                 120                 125
His Arg Asp Ile Leu Gln Ala Ser Phe Gln Asp Ser Tyr Arg Asn Leu
    130                 135                 140
Thr Leu Lys Thr Leu Ser Gly Leu Asn Trp Val Asn Lys Tyr Cys Pro
145                 150                 155                 160
Met Ala Arg Tyr Ile Leu Lys Thr Asp Asp Asp Val Tyr Val Asn Val
                165                 170                 175
Pro Glu Leu Val Ser Glu Leu Ile Gln Arg Gly Gly Pro Ser Glu Gln
            180                 185                 190
Trp Gln Lys Gly Lys Glu Ala Gln Glu Glu Thr Thr Ala Ile His Glu
        195                 200                 205
Glu His Arg Gly Gln Ala Val Pro Leu Leu Tyr Leu Gly Arg Val His
    210                 215                 220
Trp Arg Val Arg Pro Thr Arg Thr Pro Glu Ser Arg His His Val Ser
225                 230                 235                 240
Glu Glu Leu Trp Pro Glu Asn Trp Gly Pro Phe Pro Pro Tyr Ala Ser
                245                 250                 255
Gly Thr Gly Tyr Val Leu Ser Ile Ser Ala Val Gln Leu Ile Leu Lys
            260                 265                 270
Val Ala Ser Arg Ala Pro Pro Leu Pro Leu Glu Asp Val Phe Val Gly
        275                 280                 285
Val Ser Ala Arg Arg Gly Gly Leu Ala Pro Thr His Cys Val Lys Leu
    290                 295                 300
Ala Gly Ala Thr His Tyr Pro Leu Asp Arg Cys Cys Tyr Gly Lys Phe
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```
305                 310                 315                 320
Leu Leu Thr Ser His Lys Val Asp Pro Trp Gln Met Gln Glu Ala Trp
                325                 330                 335
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            340                 345                 350
Trp Leu Gln Gly Phe Leu Gly Thr Leu Arg Cys Arg Phe Ile Ala Trp
        355                 360                 365
Phe Ser Ser
    370
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<400> 29

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Gly Gly Leu Ala Phe Cys Gly Thr Thr Leu Leu Tyr Leu Ala Arg Cys
            20                  25                  30
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        35                  40                  45
Ala Lys Ala Phe Leu Ala Val Leu Val Ala Ser Ala Pro Arg Ala Val
    50                  55                  60
Glu Arg Arg Thr Ala Val Arg Ser Thr Trp Leu Ala Pro Glu Arg Arg
65                  70                  75                  80
Gly Gly Pro Glu Asp Val Trp Ala Arg Phe Ala Val Gly Thr Gly Gly
                85                  90                  95
Leu Gly Ser Glu Glu Arg Arg Ala Leu Glu Leu Glu Gln Ala Gln His
            100                 105                 110
Gly Asp Leu Leu Leu Leu Pro Ala Leu Arg Asp Ala Tyr Glu Asn Leu
        115                 120                 125
Thr Ala Lys Val Leu Ala Met Leu Thr Trp Val Asp Glu Arg Val Asp
    130                 135                 140
Phe Glu Phe Val Ile Lys Ala Asp Asp Asp Ser Phe Ala Arg Leu Asp
145                 150                 155                 160
Ala Ile Leu Val Asp Leu Arg Ala Arg Glu Pro Ala Arg Arg Arg Arg
                165                 170                 175
Leu Tyr Trp Gly Phe Phe Ser Gly Arg Gly Arg Val Lys Pro Gly Gly
            180                 185                 190
Arg Trp Arg Glu Ala Ala Trp Gln Leu Cys Asp Tyr Tyr Leu Pro Tyr
        195                 200                 205
Ala Leu Gly Gly Gly Tyr Val Leu Ser Ala Asp Leu Val His Tyr Ile
    210                 215                 220
Arg Leu Ser Arg Glu Tyr Leu Arg Ala Trp His Ser Glu Asp Val Ser
225                 230                 235                 240
Leu Gly Thr Trp Leu Ala Pro Val Asp Val Gln Arg Glu His Asp Pro
                245                 250                 255
Arg Phe Asp Thr Glu Tyr Lys Ser Arg Gly Cys Asn Asn Gln Tyr Leu
            260                 265                 270
Val Thr His Lys Gln Ser Pro Glu Asp Met Leu Glu Lys Gln Gln Met
        275                 280                 285
Leu Leu His Glu Gly Arg Leu Cys Lys His Glu Val Gln Leu Arg Leu
    290                 295                 300
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Ser Tyr Val Tyr Asp Trp Ser Ala Pro Pro Ser Gln Cys Cys Gln Arg
305                 310                 315                 320
Lys Glu Gly Val Pro
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/15452

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/70, 48/00; C07H 21/04; C12N 9/10
US CL : 424/279.1; 514/23, 44; 536/23.2; 435/183, 193
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/279.1; 514/23, 44; 536/23.2; 435/183, 193

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/49018 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 30 September 1999 (30.09.1999), see entire document.	1-29
Y	BISTRUP ET AL. Sulfotransferases of Two Specificities Function in the Reconstitution of High Endothelial Cell Ligands for L-selectin. J. Cell Biol. 17 May 1999, Vol 145. No. 4, pages 899-910, see entire document.	1-29
Y	LEE ET AL. Cloning and Characterization of a Mammalian N-Acetylglucosamine-6-sulfotransferase That Is Highly Restricted to Intestinal Tissue. Biochem. Biophys. Res. Com. 1999, Vol. 263, pages 543-549, see entire document.	1-29
Y	HIRAOKA ET AL. A Novel, High Endothelial Venule-Specific Sulfotransferase Expresses 6-Sulfo Sialyl LewisX, an L-Selectin Ligand Displayed by CD34. Immunity. July 1999, Vol 11, pages 79-89, see entire document.	1-29
Y	KIMURA ET AL. Reconstitution of functional L-selectin ligands on a cultured human endothelial cell line by cotransfection of alpha 1->3 fucosyltransferase VII and newly cloned GlcNAcbeta:6-sulfotransferase cDNA. Proc. Natl. Acad. Sci. USA. April 1999, Vol. 96, pages 4530-4535, see entire document.	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
27 June 2001 (27.06.2001)

Date of mailing of the international search report
26 JUL 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
Jessica H. Roark
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/15452

Continuation of B. FIELDS SEARCHED Item 3: WEST, MEDLINE, CAPLUS, GENBANK search terms: inventor names, MECA-79, sulfotransferase, L-selectin